BioVendor Reviews

04/2008

BIOVENDOR R&D NEWSLETTER FOCUSED ON

CLUSTERIN

BioVendor
Research
and Diagnostic Products

CLUSTERIN

Clusterin is a serum glycoprotein endowed with cell aggregating, complement inhibitory, and lipid binding properties, and is also considered as a specific marker of dying cells, its expression being increased in various tissues undergoing programmed cell death [41]. Clusterin is induced de novo during the regression of the prostate, the mammary gland and other hormone-dependent tissues after hormone ablation, and is over-expressed in several human neurodegenerative diseases including Alzheimer's disease, epilepsy and retinitis pigmentosa [45].
Clusterin is a heterodimeric glycoprotein produced by a wide array of tissues and found in most biologic fluids. A number of physiologic functions have been proposed for clusterin based on its distribution and in vitro properties. These include the above mentioned complement regulation and lipid transport, as well as sperm maturation, initiation of apoptosis, endocrine secretion, membrane protection, and promotion of cell interactions. A prominent and defining feature of clusterin is its induction in such disease states as glomerulonephritis, polycystic kidney disease, renal tubular injury, neurodegenerative conditions including Alzheimer's disease, atherosclerosis, and myocardial infarction. The expression of clusterin in these states is puzzling, from the specific molecular species and cellular pathways eliciting such expression, to the roles subserved by clusterin once induced [57].
Overall, the evidence suggests that function of clusterin is to protect surviving cells after damage. This protection may result from a detergent- and chaperon-like action of the protein [114, 105].

Clusterin has originally been purified from rat rete testis fluid by conventional techniques and by immunoaffinity chromatography. The molecule is characterized as a glycoprotein having a molecular mass of approximately 80,000 Da and an isoelectric point of 3.6. The purified protein retains the capacity to elicit clustering of cells in an in vitro assay. Under reducing conditions in the presence of sodium dodecyl sulfate, clusterin dissociates into subunits of about 40,000, designated alpha (34-36 kDa), and beta (36-39 kDa). It contains 10 cysteine residues, the numbers and locations of which are conserved in several mammalian species. All the cysteine residues are involved in interchain (alpha-beta) disulphide bonds. There are no free cysteine residues [16]. Disulfide bonds were determined between Cys58(alpha)-Cys107(beta), Cys68(alpha)-Cys99(beta), Cys75(alpha)-Cys94(beta), and Cys86(alpha)-Cys80(beta). Since there is no free sulfhydryl groups in the clusterin molecule, Cys78(alpha) and Cys91(beta) should also be linked by a disulfide bond. It is notable that all of the disulfide bonds in clusterin are not only formed by inter-chain pairing, but also appear to form an anti-parallel ladder-like structure between the two chains. The unique structure could be related to the functions of clusterin [28].When deglycosylated, the molecular mass of the alpha-subunit is 24 kDa and that of the beta subunit is 28 kDa, suggesting that approximately 30% of the mass of each subunit is carbohydrate. The amino acid compositions of clusterin alpha and beta beta are very similar; however, the sequences of the first 30-amino acid residues are distinct. Some antibodies react with both subunits whereas others with only one of them [4].

Heterogeneities in apparent molecular mass were eliminated after treatment of clusterin with neuraminidase. Gel filtration chromatography revealed that clusterin exists in dimeric and tetrameric forms under conditions of neutral pH and low salt concentrations. In the presence of 6 M urea, only the monomeric form is evident, with an apparent molecular mass of approximately 85,000 Da. Clusterin, which was found to contain 4.5% glucosamine, binds to concanavalin A-Sepharose and also to wheat germ agglutinin Sepharose [1].
Matrix-assisted laser desorption ionization mass spectrometry revealed two molecular weight species of holoclusterin (58,505 +/- 250 and 63,507 +/- 200). Mass spectrometry also revealed molecular heterogeneity associated with both the alpha and beta subunits of clusterin, consistent with the presence of multiple glycoforms. The data indicate that clusterin contains 17-27% carbohydrate by weight, the alpha subunit contains 0-30% carbohydrate and the beta subunit contains 27-30% carbohydrate. The most abundant glycoforms are bisialobiantennary without fucose and the least abundant were monosialobiantennary, trisialotriantennary with two fucose and/or tetrasialotriantennary. No evidence was found for the presence of O-linked or sulfated oligosaccharides [82].
Clusterin is initially produced as a single chain, intracellular precursor of 58 kDa which contains N-linked oligosaccharide. The precursor is converted to an intracellular 70 kDa glycoprotein, which becomes the major intracellular form of clusterin prior to secretion. Maturation of the 58 kDa precursor involves conversion of high-mannose carbohydrate to complex-type carbohydrate containing sialic acid, as well as intracellular cleavage to yield alpha and beta subunits. This cleavage event occurres at a late stage of carbohydrate modification, most likely in the trans-Golgi or a post-Golgi compartment. The

maturation and secretion of clusterin occurres rapidly, with a half-time of 30-35 min. Tunicamycin treatment of cells resulted in an unglycosylated doublet comprised of one single chain and one cleaved form of clusterin. The unglycosylated clusterin species were secreted rapidly with a half-time of 20 min. Both cleavage and secretion were independent of glycosylation [6].

Clusterin is expressed at high levels in an array of specialized cell types of adult and fetal mouse tissues and in similar cell types of human tissues. Most of these cell types are highly secretory and form the cellular interfaces of many fluid compartments. This group includes epithelial boundary cells of the esophagus, biliary ducts, gallbladder, urinary bladder, ureter, kidney distal convoluted tubules, gastric glands, Brunner's glands, choroid plexus, ependyma, ocular ciliary body, endometrium, cervix, vagina, testis, epididymus, and visceral yolk sac. Several nonepithelial secretory cell types that express high levels of clusterin also line fluid compartments, such as synovial lining cells and ovarian granulosa cells. In the context of its known biochemical properties, this expression pattern suggests that localized synthesis of clusterin serves to protect a variety of secretory, mucosal, and other barrier cells from surface-active components of the extracellular environment [34].

Clusterin has chaperone-like activity

At physiological concentrations, clusterin potently protected glutathione S-transferase and catalase from heat-induced precipitation and alpha-lactalbumin and bovine serum albumin from precipitation induced by reduction with dithiothreitol. Enzyme-linked immunosorbent assay data showed that clusterin bound preferentially to heat-stressed glutathione S-transferase and to dithiothreitol-treated bovine serum albumin and alpha-lactalbumin. Size exclusion chromatography and SDS-polyacrylamide gel electrophoresis analyses showed that clusterin formed high molecular weight complexes (HMW) with all four proteins tested. Small heat shock proteins (sHSP) also act in this way to prevent protein precipitation and protect cells from heat and other stresses. The stoichiometric subunit molar ratios of clusterin:stressed protein during formation of HMW complexes (which for the four proteins tested ranged from 1.0:1.3 to 1.0:11) is less than the reported ratios for sHSP-mediated formation of HMW complexes (1.0:1.0 or greater), indicating that clusterin is a very efficient chaperone. These results suggest that clusterin may play a sHSP-like role in cytoprotection [105].

Clusterin (i) inhibits stress-induced precipitation of a very broad range of structurally divergent protein substrates, (ii) binds irreversibly via an ATP-independent mechanism to stressed proteins to form solubilized high molecular weight complexes, (iii) lacks detectable ATPase activity, (iv) when acting alone, does not effect refolding of stressed proteins in vitro, and (v) stabilizes stressed proteins in a state competent for refolding by heat shock protein 70 (HSP70). Furthermore, it was shown that, at physiological levels, clusterin inhibits stress-induced precipitation of proteins in undiluted human serum. Clusterin represents the first identified secreted mammalian chaperone. However, other reports suggest that, at least under stress conditions, clusterin may be retained within cells to exert a protective effect. Regardless of the topological site(s) of action, the demonstration that clusterin can stabilize stressed proteins in a refolding-competent state suggests that, during stresses, the action of clusterin may inhibit rapid and irreversible protein precipitation and produce a reservoir of inactive but stabilized molecules from which other refolding chaperones can subsequently salvage functional proteins [126].

The interactions of clusterin with different structural forms of alpha-lactalbumin, gamma-crystallin and lysozyme were studied. When assessed by ELISA and native gel electrophoresis, clusterin did not bind to various stable, intermediately folded states of alpha-lactalbumin nor to the native form of this protein, but did bind to and inhibit the slow precipitation of reduced alpha-lactalbumin. Reduction-induced changes in the conformation of alpha-lactalbumin, in the absence and presence of clusterin, were monitored by real-time (1)H NMR spectroscopy. In the absence of clusterin, an intermediately folded form of alpha-lactalbumin, with some secondary structure but lacking tertiary structure, aggregated and precipitated. In the presence of clusterin, this form of alpha-lactalbumin was stabilised in a non-aggregated state, possibly via transient interactions with clusterin prior to complexation. Additional experiments demonstrated that clusterin potently inhibited the slow precipitation, but did not inhibit the rapid precipitation, of lysozyme and gamma-crystallin induced by different stresses. These results suggest that clusterin interacts with and stabilises slowly aggregating proteins but is unable to stabilise rapidly aggregating proteins. Collectively, these results suggest that during its chaperone action, clusterin preferentially recognises partly folded protein intermediates that are slowly aggregating whilst venturing along their irreversible off-folding pathway towards a precipitated protein [145].

Using sequence analyses, it was shown that clusterin likely contains three long regions of natively disordered or molten globule-like structures containing putative amphipathic alpha-helices. Natively disordered regions with

amphipathic helices form a dynamic, molten globule-like binding site provide clusterin the ability to bind to a variety of molecules [137].

Local acidosis is common at sites of tissue damage or stress.
It was found by affinity chromatography and ELISA that the binding of clusterin to glutathione-S-transferase, IgG, apolipoprotein A-I, and complement protein C9 was enhanced at mildly acidic compared to physiological pH. Analytical ultracentrifugation and gel filtration studies revealed that clusterin exists in different polymerization states with monomer occurring preferentially at pH 5.5 and multimeric species at pH 7.5. Although circular dichroism showed little difference in the alpha-helical and beta-sheet contents of clusterin at pH 5 compared to pH 7.5, evidence for pH-dependent structural changes in clusterin was obtained from fluorescence experiments. pH titrations showed reversible changes in the fluorescence of tryptophan residues in clusterin. There was a reversible 2-fold increase in the fluorescence of the extrinsic probe 4, 4'-bis(1-anilinonaphthalene-8-sulfonate) bound to clusterin at pH 5.5 compared to pH 7.5. There was also a 3.5-fold increase in fluorescence resonance energy transfer from tryptophan residues in clusterin to 4,4'-bis(1-anilinonaphthalene-8-sulfonate) at pH 5.5 compared to pH 7.5. These data suggest that pH-induced changes in the structure of clusterin are responsible for its enhanced ability to bind protein ligands at mildly acidic pH [117].

Clusterin is the first chaperone shown to be activated by reduced pH. This unique mode of activation appears to result from an increase in regions of solvent-exposed hydrophobicity, which is independent of any major changes in secondary or tertiary structure. A model was proposed in which low pH-induced dissociation of clusterin aggregates increases the abundance of the heterodimeric chaperone-active species, which has greater hydrophobicity exposed to solution [146].

Truncated, nonglycosylated, nuclear clusterin variant

In addition to the well characterized secreted form of the protein, there exists an intracellular, nuclear form of clusterin. This intracellular form of the protein was found to be induced to accumulate in the nucleus of two epithelial cell lines (HepG2 and CCL64) in response to treatment with transforming growth factor beta (TGF beta). It was demonstrated in vitro that clusterin protein can be translated from two in-frame ATG sites. Initiation from the first ATG encodes for the secretory form of clusterin and initiation from the second ATG, located 33 amino acids downstream of the first and lacking the hydrophobic signal sequence, encodes for a truncated clusterin protein. This shorter form of clusterin is not recognized by microsomes and therefore not glycosylated, and it was postulated that it is retained intracellularly and targeted to the nucleus due to the presence of an SV40-like nuclear localization sequence (NLS). This mechanism of nuclear targeting of apoJ occurs in cells since the protein isolated from nuclei of TGF beta-treated cells and the in vitro-translated truncated form are identical by V8 protease analysis. These results suggest that the diverse physiological responses attributed to clusterin may be elicited through a common molecular mechanism involving a previously uncharacterized intracellular form of the protein [65].
Nuclear clusterin (nCLU) is an ionizing radiation (IR)-inducible protein that binds Ku70, and triggers apoptosis when overexpressed in MCF-7 cells. Endogenous nCLU synthesis is a product of alternative splicing. Reverse transcriptase-PCR analyses revealed that exon II, containing the first AUG and encoding the endoplasmic reticulum-targeting peptide, was omitted. Exons I and III are spliced together placing a downstream AUG in exon III as the first available translation start site. This shorter mRNA produces the 49-kDa precursor nCLU protein. Ku70 binding activity was localized to the C-terminal coiled-coil domain of nCLU. Leucine residues 357, 358, and 361 of nCLU were necessary for Ku70-nCLU interaction. The N- and C-terminal coiled-coil domains of nCLU interacted with each other, suggesting that the protein could dimerize or fold. Mutation analyses indicate that the C-terminal NLS was functional in nCLU with the same contribution from N-terminal NLS. The C-terminal coiled-coil domain of nCLU was the minimal region required for Ku binding and apoptosis. MCF-7 cells show nuclear as well as cytoplasmic expression of GFP-nCLU in apoptotic cells. Cytosolic aggregation of GFP-nCLU was found in viable cells. These results indicate that an inactive precursor of nCLU exists in the cytoplasm of non-irradiated MCF-7 cells, translocates into the nucleus following IR, and induces apoptosis [149].
During the course of a study to examine the effect of cycloheximide on apoptosis-related genes, the variant rat clusterin mRNA deficient of the exon 5 was found. The putative protein encoded by the variant clusterin mRNA is only constituted from the N-terminal one-third portion of the ordinary clusterin protein. The expression of the variant form was increased dramatically by cycloheximide treatment, while that of the ordinary form was not affected very much. The similar phenomenon was also observed by the use of other types of protein synthesis inhibitors, anisomycin and emetine. The enhancement of expression of the variant was observed in the rat treated with heat

shock as well. The variant form was presumably generated by the exon skip mechanism. Systematic analyses of cycloheximide effect on the alternative splicing at various splicing junctions were performed. However, cycloheximide did not exhibit any remarkable effects on other types of alternative splicing, including exon skip in beta A4-amyloid protein precursor (APP) gene, alternative donor selection in Fas antigen gene and alternative acceptor selection in catechol O-methyltransferase (COMT) gene. These results indicated that the induction of exon skip by both protein synthesis inhibition and heat shock treatment occurs in a limited number of genes, if not only in clusterin [68].

A cDNA clone designated p116 was isolated from rat seminal vesicles. A sequence study suggested the expression of a transcript predicting an alternative form of clusterin. RT-PCR demonstrated the androgen-dependent expression of p116 transcript in the seminal vesicles, ventral prostate, the liver and the thymus. Since clusterin has been suggested as a classical molecular marker of apoptosis, p116 transcript newly identified in the this study might provide a useful probe to further understand the roles of clusterin and its related proteins during the course of apoptotic process not only in male accessory sex organs, but also in many types of cells undergoing apoptosis [92].

A similar variant mRNA lacking exon 5 was also induced by heat shock treatment of the human culture cell line HepG2. On the other hand, in mouse cell line L929, heat shock treatment induced a variant clusterin mRNA lacking only a small region located in exon 5. However, irrespective of the difference of mechanism of variant production, all the variant clusterin mRNA species derived from each animal species encoded a putative protein constituted from the N-terminal one-third of the clusterin protein attached to a C-terminal clusterin unrelated tail. In humans, the variant clusterin species was not detected in normal tissues but was present in certain kinds of tumour cells. These results indicate that the splicing variants were induced as a direct result of heat shock treatment on cells per se and that the phenomenon of heat shock induction was observed in culture cells derived from different animal species [84].

Clusterin can evade the secretory pathway

In live intact cells, under certain stress conditions, clusterin can evade the secretion pathway and reach the cytosol. This was demonstrated using several complementary approaches. Flow cytometry and selective permeabilization of U251 cell membranes with digitonin allowed detection of cytosolic clusterin in stressed U251 cells. In addition, a stringent enzymatic assay reliant upon the exclusively cytosolic deubiquitinase enzymes confirmed that clusterin synthesized with its hydrophobic secretion signal sequence can reach the cytosol of U251 cells. The retrotranslocation of clusterin is likely to occur through a mechanism similar to the endoplasmic reticulum (ER)-associated protein degradation pathway and involves passage through the Golgi apparatus. ER-associated ubiquitin ligase Hrd1/synoviolin can interact with, and ubiquitinate clusterin [181].

Clusterin and HDL

Clusterin was also termed apolipoprotein J (apoJ), and was purified from human plasma by immunoaffinity chromatography and found to be associated with high density lipoproteins (HDL) and specifically with subclasses of HDL which also contain apoAI and cholesteryl ester transfer protein activity [3].

Apolipoprotein J (apoJ)-containing high-density lipoproteins (HDL), isolated from human plasma by immunoaffinity chromatography, are associated with apoAI and a protein of approximately 44 kDa. The 44-kDa protein, a monomeric glycoyslated polypeptide, was identified by N-terminal sequencing as serum paraoxonase. Not all of the plasma paraoxonase is associated with apoJ. Both oxidation states of paraoxonase bind to apoJ with equal affinity. These data combined with other evidence suggest that the plasma link of apoJ with paraoxonase might be implicated as a predictor of vascular damage [40].

Purified apoJ added directly to apoJ-depleted plasma can interact with apoAI or with apoAI-containing lipoproteins, as evidenced by the association of apoAI with apoJ that is reisolated by immunoaffinity chromatography. The amount of apoAI associated with apoJ increases linearly with increasing amount of apoJ added, over the range of apoJ concentrations tested. No other known apolipoprotein is associated with apoJ. By two-dimensional electrophoretic analysis, the lipoproteins containing both apoJ and apoAI have approximate molecular masses of 350-400 kDa. Taken together, the results suggest that the interaction between apoJ and apoAI is physiologically important and that lipoproteins which contain both apoJ and apoAI can be produced in the plasma. ApoJ-HDL and apoJ/apoAI-HDL may have different functions and metabolic fates or may represent different stages of apoJ catabolism [26].

It was demonstrateh that HepG2 human hepatocellular carcinoma cells secrete apoJ in association with a significant amount of lipid, providing unequivocal evidence that apoJ can transport lipids. The HepG2 cell line

has provided important clues about the structural organization of nascent lipoprotein particles. HepG2 cell apoJ-containing lipoproteins are dense and heterogenous in size, ranging from 100 to 910 kDa. Plasma and HepG2 cell apoJ-lipoproteins differ in size distribution. Both have alpha 2 electrophoretic mobility, although their average mobilities differ within the alpha 2 region. In contrast to plasma apoJ-HDL which contain little triglyceride and which can associate with apoA-I, HepG2 cell apoJ-lipoproteins are rich in triglyceride and lack apoA-I. By implication, nascent apoJ-lipoproteins undergo plasma remodeling that results in triglyceride depletion and apoA-I association. We propose that the metabolic consequences of this remodeling play an important role in lipid homeostasis in localized tissue environments, particularly where organs are isolated from the blood by cellular barriers such as in testis and brain. In such tissues, apoJ is expressed constitutively in high level compared to other lipid transport proteins [27].

Clusterin is a positive acute phase protein

Endotoxin (LPS), tumor necrosis factor (TNF), and interleukin (IL)-1 increased hepatic mRNA and serum protein levels of clusterin in Syrian hamsters. Hepatic clusterin mRNA levels increased 10- to 15-fold with doses of LPS from 0.1 to 100 micrograms/100 g body weight within 4 h and were elevated for > or = 24 h. Serum clusterin concentrations were significantly increased by 16 h and further elevated to 3.3 times that of control, 24 h after LPS administration. Serum clusterin was associated with high density lipoprotein and increased fivefold in this fraction, after LPS administration. Hepatic clusterin mRNA levels increased 3.5- and 4.6-fold, with TNF and IL-1, respectively, and 8.2-fold with a combination of TNF and IL-1. Serum clusterin concentrations were increased 2.3-fold by TNF, 79% by IL-1, and 2.9-fold with a combination of TNF and IL-1. These results demonstrate that apo J is a positive acute phase protein [51].

Clusterin receptors

LRP-2

Clusterin has three independent classes of binding sites for 1) LRP-2, 2) Stressed proteins, and 3) Ustressed ligands, respectively, and the binding sites for LRP-2 and stressed proteins are likely to be in parts of the molecule other than the C-terminal region of the alpha-chain or the N-terminal region of the beta-chain. It has been suggested that, in vivo, clusterin binds to toxic molecules in the extracellular environment and carries these to cells expressing LRP-2 for uptake and degradation. This hypothesis is supported by the demonstration that clusterin has discrete binding sites for LRP-2 and other (potentially toxic) molecules [138].

Glycoprotein 330 (gp330), low density lipoprotein receptor-related protein-2/megalin (LRP-2) is a member of a family of endocytic receptors related to the low density lipoprotein receptor. gp330 has previously been shown to bind a number of ligands in common with its family member, the low density lipoprotein receptor-related protein (LRP). To identify ligands specific for gp330 and relevant to its localization on epithelia such as in the mammary gland, gp330-Sepharose affinity chromatography was performed. As a result, a 70-kDa protein was selected from human milk and identified by protein sequencing to be apolipoprotein J/clusterin (apoJ). Solid-phase binding assays confirmed that gp330 bound to clusterin with high affinity (Kd = 14.2 nM). Similarly, gp330 bound to clusterin transferred to nitrocellulose after SDS-polyacrylamide gel electrophoresis. LRP, however, showed no binding to clusterin in either type of assay. The binding of gp330 to clusterin could be competitively inhibited with excess clusterin as well as with the gp330 ligands apolipoprotein E, lipoprotein lipase, and the receptor-associated protein- a 39-kDa protein that acts to antagonize binding of all known ligands for gp330 and LRP. Several cultured cell lines that express gp330 and ones that do not express the receptor were examined for their ability to bind and internalize 125I-clusterin. Only cells that expressed gp330 endocytosed and degraded radiolabeled clusterin. Furthermore, F9 cells treated with retinoic acid and dibutyryl cyclic AMP to increase expression levels of gp330 displayed an increased capacity to internalize and degrade clusterin. Cellular internalization and degradation of radiolabeled clusterin could be inhibited with unlabeled clusterin, receptor-associated protein, and gp330 antibodies. The results indicate that gp330 but not LRP can bind to apoJ in vitro and that gp330 expressed by cells can mediate clusterin endocytosis leading to lysosomal degradation [56].

After endocytosis, the LRP-2 is recycled back to the cell surface while clusterin is delivered to the lysosomes for degradation. To provide additional evidence implicating LRP-2 in clusterin endocytosis, a receptor-associated protein (RAP), an antagonist of apo J binding to LRP-2, was injected into the efferent duct lumen. Subsequent immunocytological analysis of the efferent duct showed that the RAP treatment abolished the endocytosis of apo J by the nonciliated cells. Taken together, these data indicate that LRP-2 is a likely mediator of apo J endocytosis by the nonciliated efferent duct cells [69].

TGF-betaRI and II

Clusterin interacts with both the type I (RI) and type II (RII) TGF beta receptors but does not interact with the epidermal growth factor (EGF) receptor. The interaction between RII and clusterin occurs through the C-terminal 127 amino acids of RII. Deletion of this region, which contains the kinase insert 2 domain, abrogates binding to clusterin. The binding of clusterin to either the RI and the RII receptors is direct, not requiring other proteins, and is not specific for the alpha or beta subunit of apoJ since both subunits are effective in competing for binding. RI and RII fusion proteins are capable of precipitating the 60 kDa intracellular form of apoJ from [35S]methionine-labeled cellular lysates, suggesting that this form of the protein may play some role in TGF beta signaling or TGF beta receptor processing [61].

Clusterin is also a modulator of TGF-beta signaling by regulating Smad2/3 proteins. Overexpression of clusterin enhanced TGF-beta-induced transcriptional activity and increased the amount of Smad2/3 proteins, while clusterin siRNA repressed TGF-beta-induced transcriptional activity and decreased the amount of Smad2/3 proteins in Hep3B cells. Clusterin is also involved in Smad2/3 stability at the protein level. These findings suggest that CLU regulates TGF-beta signaling pathway by modulating the stability of Smad2/3 proteins [190].

CLUSTERIN REGULATION

Both a transient heat shock (20 min at 42 degrees C) and various oxidative stresses, including hydrogen peroxide, superoxide anion, hyperoxia and UVA exposure, induce a strong increase in clusterin mRNA levels as assessed by northern blot. Nuclear run-on analysis suggests that transcriptional activation is involved in inducing clusterin mRNA in response to heat shock. Using pulse-chase analysis of control and heat shocked cells, it is shown that clusterin mRNA is translated and secreted, thus resulting in increased extracellular levels of the protein following heat shock. To investigate the function of clusterin in response to these stresses, clusterin anti-sense transfectants that stably express virtually no clusterin at the mRNA and protein level were generated in A431 cells. These anti-sense transfectants are shown to be highly sensitive to apoptotic cell death induced by heat shock or oxidative stress compared with wild-type A431 cells or control transfectants. Taken together, these results show that clusterin gene expression is induced in response to heat shock and oxidative stress in human A431 cells, and confers cellular protection against heat shock and oxidative stress [106].

Clusterin gene and promoter

The only DNA region strictly conserved between clusterin gene proximal promoters from different vertebrate classes is a 14-bp DNA element which is specifically recognized by the HSF1 (and HSF2169) transcription factor and which can mediate heat-shock-induced transcription in transient expression assays. Conversely, the avian clusterin proximal promoter, point-mutated at the level of this element, no longer transmits heat-shock activation. These findings provide a possible explanation for the high sensitivity of clusterin expression to environmental changes and allow the classification of clusterin as an extracellular version of heat-shock protein [85].

The gene encoding rat clusterin was isolated and characterized, and its cytosine methylation pattern in various tissues was analyzed. Several putative regulatory DNA elements were identified, including a consensus AP-1 site in the 5‘ flanking region. Two AP-1 sites and two transforming growth factor-beta inhibitory elements, one AP-2 site and eight half-sites for glucocorticoid/androgen response elements were found within the first intron, and one cAMP response element was found in the first exon. The cytosine methylation pattern indicated that testicular or epididymal DNA in the rat is hypomethylated in the region between positions -534 and -99 of the clusterin gene, when compared with tissues with lower levels of expression such as prostate as well as liver, lung, kidney and spleen [49].

Human clusterin gene contains a Myb binding site in its 5‘ flanking region, which interacts with bacterially synthesized B-MYB protein and mediates B-MYB-dependent transactivation of the clusterin promoter in transient transfection assays. Endogenous clusterin expression is induced in mammalian cell lines following transient transfection of a B-MYB cDNA. Blockage of secreted clusterin by a monoclonal antibody results in increased apoptosis of neuroblastoma cells exposed to the chemotherapeutic drug doxorubicin. Thus, activation of clusterin by B-MYB may be an important step in the regulation of apoptosis in normal and diseased cells [119].

The Wnt signaling pathway specifically regulates one out of three clusterin mRNA variants via TCF1. This clusterin transcript is shorter at the 5‘ end than reported by the RefSeq database, and produces the intracellular 60 kDa CLU protein isoform which is secreted as a ~80 kDa protein after post-translational processing [184].

TGFbeta

TGFbeta stimulates both clusterin mRNA and protein levels, and induces its accumulation in the nucleus of CCL64 cells. A 1.3-kilobase rat clusterin promoter/luciferase reporter construct was created and it was demonstrated that TGFbeta enhances luciferase activity 2.5-6-fold in transient transfection assays of epithelial, endothelial, and fibroblast cell lines. Deletional analysis reveals that an AP-1-binding site (5'-TGAGTCA) in the minimal promoter region is necessary for initiating transactivation by TGFbeta. A single T to G base mutation in the AP-1 site (5'-TGAGGCA) abolishes TGFbeta-induced clusterin promoter transactivation. In transcription factor decoy experiments, 23-mer oligonucleotides of wild type AP-1 reduce TGFbeta induction of clusterin mRNA levels and promoter transactivation, while an oligonucleotide containing the mutated AP-1 site has no effect. Two specific protein kinase C inhibitors, GF109203X and calphostin C, block TGFbeta-induced clusterin mRNA levels and promoter transactivation. Together these results indicate that TGFbeta regulates clusterin gene expression through an AP-1 site and its cognate transcription factor AP-1, and requires the involvement of protein kinase C [81].

OX-PAPC and Interleukin-6

Treatment of HepG2 cells with oxidized 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphoryl choline (OX-PAPC), or biologically active lipids present in mildly oxidized low density lipoprotein, increased clusterin, and decreased paraoxonase (PON) mRNA levels. Antibodies to IL-6 blocked these changes. IL-6 treatment in the absence of OXPL produced the same pattern of mRNA changes observed with OXPL treatment alone. In vivo, OX-PAPC injected into C57BL/6J mice resulted in a marked reduction in PON activity and an increase in clusterin levels in plasma after 16 h. Injection of OX-PAPC into IL-6-deficient C57BL/6J mice (IL-6 -/-) did not alter either PON activity or apoJ levels [123].

Glucocorticoids

In the kidney-derived epithelial cell line MDCK, clusterin mRNA is repressed by glucocorticoids and by progesterone. Treatment with epidermal growth factor also represses clusterin gene expression in MDCK cells. Incubation with 12-O-tetradecanoyl-phorbol-13-acetate, which activates protein kinase C (PKC), induces clusterin mRNA, while chelerythrine, an inhibitor of PKC, represses clusterin gene expression, suggesting that the clusterin gene responds to signalling pathways involving PKC [73].

Lactogenic Hormones

The effect of lactogenic hormones and EGF on the expression of involution-induced genes in HC11 mammary epithelial cells was studies. Insulin, dexamethasone, prolactin, and its combinations did not affect expression of the genes. When cells were cultured in growth medium containing EGF the expression of clusterin was strongly inhibited in a dose- and time- dependent manner [83].

Angiotensin II

Angiotensin II suppression of clusterin expression in the liver of hypertensive rats may represent a specific response to high levels of circulating angiotensin II or a response to hypertensive injury [100].

Testosterone

Testosterone repressed clusterin gene expression is rapidly induced in early involution of the mouse mammary gland, after weaning, and in the rat ventral prostate, after castration. A search for involution-enhanced DNaseI footprints in the proximal mouse clusterin gene promoter led to the identification and characterization (by DNase I footprinting and EMSA) of a twin nuclear factor 1 (NF1) binding element at -356/-309, relative to the proposed transcription start site; nuclear extracts from 2-day involuting mouse mammary gland showed an enhanced footprint over the proximal NF1 element; extracts from involuting prostate showed enhanced occupancy of both NF1 binding elements. Subsequent EMSA and Western analysis led to the detection of a 74-kDa NF1 protein whose expression is triggered in early involution in the mouse mammary gland; such an induced protein is not found in the involuting rat ventral prostate. This protein was not found in lactation where three other NF1 proteins of 114, 68, and 46 kDa were detected. Reiteration of the epithelial cell apoptosis associated with early mammary gland involution, in vitro, in a primary cell culture system, triggered the appearance of the 74-kDa NF1. Overlaying the cells with laminin-rich extracellular matrix suppressed the apoptosis and the expression of the 74-kDa NF1 and, in the presence of lactogenic hormones, initiated milk protein gene expression and the expression of two of the lactation-associated NF1 proteins (68 and 46 kDa). This study, thus, identifies the occurrence of a switch in expression of different members of the family of NF1 transcription factors as mammary epithelial cells move from the differentiated to the involution/apoptotic state, and it is likely that the involution-specific 74-kDa NF1 accounts for the enhanced NF1 footprint detected on the clusterin promoter with extracts of mouse mammary gland [75].

To investigate whether endocrine disruptors affect spermatogenesis through an clusterin-dependent mechanism, daily oral doses of testosterone (50, 200 and 1,000 microg/kg), flutamide (1, 5 and 25 mg/kg), ketoconazole (0.2, 1, 5 and 25 mg/kg), diethylhexylphthalate (10, 50 and 250 mg kg), nonylphenol (10, 50, 100 and 250 mg/kg), octylphenol (10, 50 and 250 mg kg), diethylstilbesterol (10, 20 and 40 microg/kg) or corn oil (control) were administered to 5 week-old, male Sprague-Dawley rats for 3 weeks. Following treatment with these endocrine disruptors, testicular expression of clusterin mRNA was analyzed using reverse transcription-polymerase chain reaction. Compared with the control, the lowest dose of testosterone (50 microg/kg/day) significantly increased expression of clusterin mRNA, whereas 200 and 1,000 microg/kg/day testosterone significantly decreased the expression ($P<0.05$). Flutamide, ketoconazole, diethylhexylphthalate, nonylphenol, octylphenol and diethylstilbesterol significantly decreased clusterin mRNA expression in testes at all doses studied, with the exception of 1 mg/kg/day flutamide ($P<0.05$). These results suggest that endocrine disruptors might decrease spermatogenesis in testes by decreasing expression of clusterin mRNA [183].

Estrogens

Clusterin mRNA expression in rat endometrial adenocarcinoma cells is tightly regulated by estrogens and anti-estrogens in vitro and in vivo, and there is a complex mechanism of regulation of clusterin expression in the normal and cancerous endometrium [99].

Vitamin A

In vitamin A-deficient (VAD) rats before and after administration of all-trans retinoic acid (ATRA), genes, the transcription of which was influenced by ATRA, were isolated. One gene was isolated, the transcription of which was reduced to about 70% by ATRA- clusterin. The effect of ATRA on clusterin expression may be direct, since the promoter of Sgp-2 contains a putative ATRA-responsive element (RARE) [94].

TSH

A prominent secretory glycoprotein was detected in the culture medium of porcine thyrocytes which was identified as clusterin by microsequencing. Treatment of thyrocytes with thyroid stimulating hormone revealed a tight regulation of both synthesis and secretion of clusterin, with a distinct fraction of clusterin being always associated with the cells [115].

Thyroid Hormones

Clusterin was found to be down-regulated by thyroid hormones in HepG2 cell line [158].

Thrombin

Thrombin (10(-8) M) increased clusterin mRNA levels two- to fourfold in glomerular mesangial, glomerular epithelial, and proximal tubule epithelial cells. This was a specific effect of thrombin receptor activation because peptides corresponding to the tethered ligand of the thrombin receptor were also able to increase clusterin mRNA levels. Epidermal growth factor, insulin-like growth factor-1, and transforming growth factor-beta 1 had little or no effect on clusterin mRNA levels. The protein kinase C inhibitor RO-32-0432 (1 microM) inhibited the thrombin-induced increase in clusterin mRNA, suggesting that thrombin receptor activation may regulate renal clusterin mRNA levels through protein kinase C [78].

APOPTOSIS

It has been shown that clusterin expression is only confined to surviving cells following the induction of apoptosis in vitro, suggesting that it is involved in cell survival rather than death [106].On the basis of its elevated expression in apoptotic tissues, it was originally proposed that the protein might be casually involved in apoptosis. In contrast to the earlier notion, clusterin expression is not enhanced, but rather is down-regulated in the cells undergoing apoptosis and that its expression in the apoptotic tissue is restricted to the vital neighboring cells. These results led to the proposal that rather than being a cell death gene, clusterin is a cell survival gene, exerting a protective function on the surviving bystander cells [61]. It was suggested that clusterin gene induction in the vital cells is produced by signaling molecules that are generated by the apoptotic cells and that apoptotic processes [108]. Exposure of vital fibroblasts to apoptotic vesicles, disrupted vital cells, and trypsin-treated membrane remnants induces clusterin mRNA. Moreover, lipid vesicles consisting of phosphatidylserine (PtSer) and dimyristoylphosphatidylcholine (PC), but not liposomes with PC alone nor with dimyristoylphosphatidylethanolamine or phosphatidic acid, did elevate clusterin mRNA level. These results suggest that, apart from mediating the endocytic uptake of the apoptotic vesicles, PtSer also serves as a trigger to stimulate the expression of genes that might be involved in the cellular clearance process [131].

Intracellular clusterin inhibits apoptosis by interfering with Bax activation in mitochondria. Intriguingly, in contrast to other inhibitors of Bax, clusterin specifically interacts with conformation-altered Bax in response to chemotherapeutic drugs. This interaction impedes Bax oligomerization, which leads to the release of cytochrome c from mitochondria and caspase activation. Moreover, we also find that clusterin inhibits oncogenic c-Myc-mediated apoptosis by interacting with conformation-altered Bax. Clusterin promotes c-Myc-mediated transformation in vitro and tumour progression in vivo. Taken together, these results suggest that the elevated level of clusterin in human cancers may promote oncogenic transformation and tumour progression by interfering with Bax pro-apoptotic activities [168].

Biological effects of intracellular clusterin were studied and it was observed observed that clusterin forms containing the alpha-chain region strongly accumulated in an ubiquitinated form in juxtanuclear aggregates meeting the main criterions of aggresomes and leading to profound alterations of the mitochondrial network. The viability of cells transfected by intracellular forms of clusterin was improved by overexpression of Bcl-2, and caspase inhibition was capable of rescuing cells expressing clusterin, which presented an altered mitochondrial permeability. It is propose that, although it might be an inherently pro-survival and anti-apoptotic protein expressed by cells under stress in an attempt to protect themselves, clusterin can become highly cytotoxic when accumulated in the intracellular compartment [151].

Confocal microscopy demonstrates that the nuclear accumulation of clusterin is coincident with DNA fragmentation. These data suggest that, at least in secretory epithelial cells, retrograde transport from the Golgi to the ER of a nonglycosylated, uncleaved isoform and the subsequent translocation of clusterin to the nucleus occur in dying cells [156].

Accumulation of clusterin mRNA, whose rate of expression has been associated to the processes of programmed cell death induced by dexameasone in rat thymocytes, preceded the appearance of DNA degradation, starting to increase as early as 30 min after steroid injection, and maintained higher than controls until 8 hrs [5].

A number of inducible leucocyte models of apoptosis was examined, including glucocorticoid and calcium ionophore induced thymocyte death, 'aged' neutrophils and cytotoxic T cells, and it was found that, in these situations, cell death is not associated with up-regulation of clusterin gene expression. The finding that clusterin is not expressed in all cells undergoing apoptosis would suggest that this molecule is not critical to the mechanism of cell death. It does, however, provide the first example of a readily detectable marker which is differentially expressed in cells undergoing apoptosis and adds further weight to the argument that apoptosis is not a uniform phenomenon, but is dependent on the nature of the cells involved and the means of induction [31]. Another study reports that the expression of clusterin mRNA was confined to cells present in the thymic medulla, concentrated mainly around Hassal's bodies raising the possibility that the secreted protein is involved in the disposal of cell debris resulting from thymocyte apoptosis [30]. The expression of this gene in the rat thymus after the induction of thymocyte programmed cell death (PCD) by in vivo dexamethasone administration was studied. Northern blot analysis of clusterin mRNA 2, 4, 6 and 8 hr after dexamethasone administration in a total of 21 rats revealed no modification in the level of clusterin gene expression. In situ hybridization demonstrated that clusterin gene expression is macroscopically confined to the medullary region of the thymus, and that this distribution is not modified by dexamethasone administration. These results strongly suggest that in the rat, clusterin gene expression is not associated with the programmed cell death of thymocytes following in vivo dexamethasone administration [46]. Rat thymocytes were treated with dexamethasone and clusterin mRNA expression was analyzed by semi-quantitative RT-PCR before and after induction of apoptosis. Interestingly, neither the treatment with dexamethasone in vitro nor triggering of apoptosis in vivo up- regulated clusterin expression, opposing the view that clusterin is involved in apoptotic processes [150].

To assess the relationship between cell proliferation and cell death, the mRNA accumulation of ornithine decarboxylase (ODC) and clusterin were measured in human peripheral blood lymphocytes (HPBL) 2-6 hours after stimulation with phytohemagglutinin (PHA). ODC is the rate limiting enzyme of polyamines biosynthesis and its early induction in mitogen-stimulated lymphocytes has been reported. On the other hand, clusterin, a glycoprotein present in most mammalian tissues, is induced in classical models of apoptosis, such as dexamethasone-treated thymocytes. Indeed, a consistent amount of clusterin mRNA in quiescent HPBL, an early and progressive decrease of clusterin mRNA and a parallel increase of ODC mRNA accumulation, were observed, in PHA-stimulated HPBL, suggesting that concomitant repression of clusterin and induction of ODC genes contribute for the cell entering the cell cycle [14].

A single administration of the inhibitor of protein synthesis cycloheximide results in the occurrence of apoptosis in rat liver. The presence of intracellular apoptotic bodies was detected as early as 2 hours after treatment. No evidence of cell necrosis could be observed by histologic and biochemical analysis. Apoptosis was followed by an increased expression of clusterin RNA, a gene whose activity has been associated to apoptotic cell death in involuting rat prostate. The finding of in vivo induction of apoptosis in nonproliferating cells by an inhibitor of protein synthesis, together with the rapidity and synchrony in the occurrence of cell death make this model potentially useful for the analysis of the kinetics of the apoptotic cycle and in exploring some of the mechanisms of regulation of genes possibly involved in this type of cell death [17].

In the ovary, PMSG treatment alone (48 h), and in combination with hCG, dramatically reduced clusterin mRNA to 12-27% of controls (P less than 0.01). Clusterin levels were not elevated until 7 days after hCG; levels then remained constant through day 14 of pseudopregnancy. Since luteal progesterone secretion begins to diminish 5-7 days after hCG, the increased expression of Clusterin on day 7 may be related to the initiation of the regression/degeneration of luteal cells which occurs during luteolysis. Thus, this study demonstrates that alterations in Clusterin expression by the ovary may precede or occur simultaneously with cellular events initiating luteolysis and suggests a role for this glycoprotein as an early marker for luteolysis and implicates it in yet another instance of programmed cell death [20].

Using three different models of PCD: (a) ultraviolet B (UV-B) irradiation of human U937, HeLa, and A431 cell lines, (b) in vitro aging of human peripheral blood neutrophils (PMNs), and (c) dexamethasone-induced cell death of the human lymphoblastoid cell line CEM-C7, it was shown that the classical morphological and biochemical features of PCD observed did not correlate with an increase, but with either a marked decrease or an absence of clusterin gene expression as assessed by Northern blot analysis. In situ hybridization of U937 and A431 cells after UV-B irradiation revealed, in addition, that only morphologically normal cells that are surviving continue to express the clusterin gene. These results demonstrate that in the human myeloid, lymphoid, and epithelial cell types studied, clusterin gene expression is not a prerequisite to their death by apoptosis. In addition, and most interestingly, in situ hybridization of U937 and A431 cells revealed that only surviving cells express the clusterin gene after the induction of PCD, thus providing novel evidence suggesting that clusterin may be associated with cell survival within tissues regressing as a consequence of PCD [41].

A study study was carried out in an attempt to establish the function of clusterin in programmed cell death using tumor necrosis factor (TNF) alpha-induced cytotoxicity in LNCaP cells as the model system. LNCaP is an androgen-sensitive, human prostatic cancer cell line that responds to TNF in culture by undergoing programmed cell death, as determined by the loss of cell number, failure to exclude trypan blue, detection of DNA fragmentation, and increased release of previously incorporated [3H]thymidine. Immunocytochemical staining for clusterin was weak but evident in LNCaP cells. Following treatment with TNF alpha, there was a time-dependent increase in clusterin staining, the intensity of which peaked at 2 h and declined thereafter. clusterin staining in LNCaP cells was undetectable prior to the onset of DNA fragmentation at 6 h of TNF treatment. This observation indicated that TNF-induced cell death in LNCaP cells was characterized by an initial transient elevation of clusterin, followed by a period of clusterin depletion that preceded cell death. Transfection of LNCaP with a 21-base oligonucleotide antisense to clusterin resulted in a significant increase in cell death that was sequence specific and was accompanied by a reduction in clusterin biosynthesis. These findings supported the concept that clusterin depletion, rather than its expression, was associated with cell death. Finally, stable transfection and subsequent overexpression of clusterin in LNCaP cells resulted in resistance to the cytotoxic effect of TNF. These results have provided evidence to indicate that clusterin plays a role in the protection of TNF-induced cell death in LNCaP cells [55].

LNCaP cells are highly sensitive to the cytotoxic effect of TNF, while PC3 cells are resistant to TNF at 24 hr. Cells were cultured in the presence or absence of TNF (10 ng/ml). LNCaP cells were treated with varying concentrations of exogenous SGP-2, while PC3 cells were treated with antisera to SGP-2 with and without exogenous SGP-2. Following a 24-hr treatment, cultures were assessed by counting of cell number and by the trypan blue exclusion assay. Western blot analysis of conditioned media revealed that PC3 secreted more SGP-2 than did LNCaP. The sensitivity to TNF in LNCaP cells was reduced by the addition of exogenous SGP-2. PC3 cells became sensitive to TNF when SGP-2 antibody was added to the culture. The effect of SGP-2 antibody on PC3 cells was reversed by the addition of exogenous SGP-2 to the culture. CONCLUSIONS: These results suggest that SGP-2 can act as an extracellular mediator of anti-TNF-induced cytotoxicity [109].

High levels of clusterin expression, about 0.2 pg clusterin secreted per cell per 48 h period, specifically protected L929 cells from TNFalpha-mediated cytotoxicity, while low expression (about 4 fg/cell/48 h) had no effect. However, clusterin expression did not provide transfected L929 cells with protection against death mediated by colchicine, staurosporine or azide. High level expression of clusterin in transfected L929 cells also potentiated the cytotoxicity of TGFbeta. It had previously been shown that exposure of L929 cells to TGFbeta provides protection against TNFalpha. This showed that this protective effect is not additive to that of clusterin expression. One interpretation of this data is that it suggests that clusterin and TGFbeta may act via a common mechanism to provide protection against the cytotoxicity of TNFalpha. These results indicate that an intracellular action of clusterin protein is responsible for protection against TNFalpha cytotoxicity. Exposure to TNFalpha induces an increase in the level of cell-associated clusterin and specifically in the level of a novel clusterin molecule, which when analyzed under reducing conditions by SDS/PAGE and immunoblotting appears as two closely spaced bands at about 36 and 38.5 kDa. When analyzed under the same conditions, the normal form of intracellular clusterin, which is present with or without exposure to TNFalpha, appears as two poorly resolved bands at about 43-45 kDa. Since the novel form of clusterin is also expressed in cells exposed to TGFbeta, colchicine, staurosporine, and azide, it may result from toxin-induced disruption of processes of normal cellular protein production [86].

A double-stable prostatic cell line was established for inducible clusterin by using the Tet-On gene expression system. 50% of the cells over expressing clusterin escaped from TNF and actinomycine D induced cell death. Moreover, the incubation of MLL cells with conditioned medium containing the secreted clusterin or the supplementation of purified clusterin in the extracellular medium decreased significantly the TNF-induced apoptosis. This extracellular action implicates megalin, the putative membrane receptor for clusterin to mediate survival. Indeed clusterin over expression up regulated the expression of megalin and induced its phosphorylation in dose dependent manner. We interestingly showed that clusterin over expression is associated with the up-regulation of the phosphorylation of Akt. Activated Akt induced the phosphorylation of Bad and caused a decrease of cytochrome c release. These results enable us to pinpoint one mechanism by which secreted clusterin favours survival in androgen independent prostate cancer cells, implicating its receptor megalin and Akt survival pathway [192].

Apoptotic elimination of intestinal cells following irradiation has been studied in the small and large intestine of the rat, and correlated with the level of expression and localization of clusterin mRNA. Clusterin was moderately expressed in normal intestine where only small levels of apoptosis were found. After irradiation, however, there was a temporal correlation between an increased apoptotic index and increased clusterin expression. Localization of clusterin mRNA by in situ hybridization identified extensive labelling in the lower part (Paneth cell region) of small intestinal crypt, whereas epithelial cells in the large intestine were diffusely labelled. Clusterin expression was not localized over apoptotic cells and its role may be as a cell protection factor for surviving cells, as had been suggested by others. Clusterin may also be involved in remodelling of the intestinal crypt after radiation damage, a process that includes altering cell-to-cell contact, apoptosis, and sloughing of the dead cells from the intestinal villi. These results do show a close temporal link between apoptosis and clusterin expression, and, as such, expression of the gene may be a useful indicator of presence of apoptosis in the irradiated intestine [64].

As was shown that within these tissues clusterin is expressed in the surviving rather than in the dying cells, and that clusterin gene expression is actually down-regulated in the apoptotic cells, a study examined the expression of the clusterin gene in apoptotic MDCK cells. Cell death was initiated by three different stimuli: application of the steroid hormone antagonist RU 486, activation of protein kinase C by the application of the phorbol ester TPA, and--since clusterin is involved in lipid and cholesterol transport--perturbation of cell membranes by cholesterol. It was shown that clusterin gene expression was repressed in cells undergoing apoptosis in response to the application of RU 486 and TPA, but was unchanged in cells in which apoptosis has been triggered by cholesterol treatment [59].

40 archival radical prostatectomy and/or biopsy specimens of varying grades of prostate cancer were subjected to immunohistochemical SGP-2 staining. The resulting epithelial stains were quantified subjectively on a scale of 1-3 by four independent observers. Benign prostatic epithelial cells from young donors served as controls and showed a consistently weak staining intensity. In contrast, prostate cancer specimens showed varying degrees of staining intensity that correlated with a Gleason pattern ($P = 0.006$). This correlation supports the hypothesis that protection from apoptotic death may account, in part, for biologically aggressive tumor behavior [104].

Complement, Inflammation and Sepsis

Clusterin/human complement lysis inhibitor (CLI) is incorporated stoichiometrically into the soluble terminal complement complex and inhibits the cytolytic reaction of purified complement components C5b-9 in vitro10. The presence of <10% of the circulating clusterin in its heterodimeric, active form could reduce the rate of complement cytolysis of nucleated cells by 10-fold, and under some conditions by 100-fold or more. This would provide a high level of protection for certain cells and may allow time for action by other inhibitors of complement [79].

However, a conradictory study reports that clusterin gave dose-dependent protection of antibody-coated sheep erythrocytes against complement-mediated lysis by diluted normal human serum. There was a linear relationship between the concentration of clusterin giving 50% protection and the concentration of serum; extrapolation of this to the case of undiluted human serum showed that a clusterin concentration at least two orders of magnitude greater than its physiological plasma concentration would be needed to confer protection against complement-mediated cytolysis under physiological conditions. Physiological concentrations of clusterin did not protect rabbit erythrocytes against alternative complement pathway-mediated lysis using dilute human serum. Exogenous clusterin had no effect on lysis of human erythrocytes triggered by the addition of inulin to autologous human serum. Induction of cell-surface clusterin expression by L929 (murine fibroblast) cells which had been stably transfected with cDNA for human clusterin linked to DNA coding for the 44 C-terminal amino acid residues of CD55 did not protect the cells against complement-mediated lysis by either normal or clusterin-depleted human serum. These data suggest that clusterin may not be a physiologically relevant regulator of complement activation [111].

It seems that clusterin has a particular affinity for membráně attack komplex (MAC) which is associated with immunoglobulin. This observation should help to distinguish between the different forms of MAC, and might indicate that MAC associated with immunoglobulin is essentially in its cytolytically inactive form [25].

The amount of clusterin and vitronectin on MAC in plasma, also referred to as soluble MAC (SMAC) was measured, as well as on MAC bound to CIC (MAC-CIC), using antibody directed to polymerized C9 in systemic lupus erythematosus (SLE) patients. A strong correlation among the quantities of SMAC and MAC-CIC was observed. The amount of both clusterin and vitronectin associated with MAC-CIC was two- to threefold higher in comparison to the SMAC. Patients with high levels of clusterin and vitronectin demonstrated renal involvement. It was hypothesized that these complement regulatory proteins besides regulating the insertion of MAC play other critical roles, in disease pathogenesis [176].

An ELISA method for measuring the binding of clusterin to immunoglobulins was developed. Clusterin purified by IgG affinity chromatography bound to pooled human IgG with a similar affinity (S0.5 5.9 +/- 0.4 micrograms/ml) as clusterin purified by monoclonal antibody chromatography (S0.5 6.1 +/- 0.2 micrograms/ml). The apparent affinity of clusterin for IgG immobilised on ELISA plates increased with increasing concentrations of IgG in the coating solution. Aggregated IgG in solution was a more potent inhibitor of the binding of clusterin to immobilised IgG than was monomer IgG. Clusterin bound to all of the isotypes of human IgG, and to human IgA and IgM, with apparent affinities in the order IgG3 > IgG4 > IgM > IgG1 > IgG2, IgA. Clusterin bound to both the Fab and Fc fragments of human IgG. The clusterin binding site(s) on the Fc do not overlap with those for protein A and Clq [29].

C1q is responsible for the ability of clusterin preparations to promote immune complex formation [74].

The presence of complement regulators such as clusterin in Sjoegren syndrome patients‘ saliva and the high expression of these proteins in inflamed salivary gland tissue followed the inflammatory reaction. These regulators may be involved in protecting the exocrine glands from complement mediated injury [90].

Various S. aureus strains were examined and all appeared to bind clusterin to some extent, while nonpathogenic control strains Bacillus subtilis BR151 and Escherichia coli JM109 did not. Three S. aureus isolates were selected for more detailed study; binding of labeled clusterin was saturable, inhibited in the presence of excess unlabeled clusterin, and prevented by pretreatment of bacteria with proteases. From the saturation binding studies, estimates of the affinity constants for the binding of clusterin to the bacteria ranged from 31 to 57 nM. Addition of clusterin to S. aureus cultures was also found to result in aggregation of the bacterial cells; aggregation was not detected when clusterin was added to B. subtilis BR151 or E. coli JM109 cultures. These results

suggest that at least some S. aureus strains possess specific proteinaceous receptors for clusterin. Such receptors may be an important new bacterial virulence determinant for S. aureus, as clusterin has been proposed to have a role in the regulation of complement activity [72].

For clusterin, erythrocytes lysis was measured to reflect the end product of complement activation (membrane attack complex). The complement-induced hemolysis increased when human serum was pre-incubated with clusterin-binding strains, S. epidermidis J9P. The enhancement of hemolysis by J9P decreased when serum was supplemented by exogenous clusterin. The data imply that interaction between coagulase-negative staphylococci and clusterin interferes with one of their physiological functions, complement inhibition.

Vascular leakage and shock are the major causes of death in patients with dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). It has been suggested that patients with an elevated level of the free soluble form of dengue virus (DV) nonstructural protein 1 (sNS1) are at risk of developing DHF. To understand the role of sNS1 in blood, a study searched for the host molecule with which NS1 interacts in human plasma by affinity purification using a GST-fused NS1. Clusterin, which naturally inhibits the formation of terminal complement complex (TCC), was identified by mass spectrometry. A recombinant sNS1 produced from 293T cells and sNS1 from DV-infected Vero cells interacted with human Clu. Since an activated complement system reportedly causes vascular leakage, the interaction between sNS1 and Clu may contribute to the progression of DHF [185].

The aim of another study was to investigate potential protective effects of CLU on pulmonary vasculature after in situ polymorphonuclear (PMN) activation in isolated rabbit lungs. The experiments were performed on 24 isolated and ventilated rabbit lungs that were perfused with 200 mL of Krebs-Henseleit-10% blood buffer with a constant flow of 150 mL/min in a recirculating system. It was tested whether pretreatment with clusterin (2.5 microg/ml; n = 8) or catalase (CAT, 5000 U/ml; n = 8) before N-formyl-Met-Leu-Phe (fMLP; 10(-8) M) injection influenced pulmonary artery pressure (PAP) peak airway pressures (PAW) and edema formation as compared with controls (n = 8). Baseline values of PAP were 9-11 mmHg and PAW 11-13 cm H2O. Application of fMLP resulted in an acute significant ($P < 0.01$) increase of PAP (48 +/- 29 mmHg) within 2 min in the control group and PAW increased to 35 +/- 7 cm H2O within 30 min. Pretreatment with CLU completely suppressed the PAP and PAW response as a result of the fMLP challenge ($P < 0.001$), whereas a transient PAW increase up to 27 +/- 15 mmHg was observed after CAT. Complement factor C3a release was suppressed by CAT, whereas clusterin blocked the complement cascade at the level of C5b-9 formation. Moreover, generation of thromboxane A(2) was reduced after clusterin and CAT. Lung edema occurred in the fMLP group but was absent ($P < 0.001$) after clusterin and CAT treatment. Both clusterin and CAT prevented fMLP-induced lung injury. Stabilizing effects of clusterin, point towards complement regulating features at the level of the terminal complement sequence. Elevated levels of clusterin during inflammation could reflect a compensatory organ protective mechanism. Further studies are required to elucidate the clinical impact of the observed organ-protective properties of clusterin [155].

CANCER

The acquisition of resistance to a wide variety of proapototic stimuli was initially demonstrated by introducing the clusterin gene into prostate cancer cells. Furthermore, silencing clusterin expression using antisepse oligodeoxynucleotides (AS ODN) synergistically enhanced the effects of several conventional therapeutic modalities through the effective induction of apoptosis in prostate cancer xenograft models. Based on these outcomes, Phase I clinical trials were conducted using AS clusterin ODN incorporating 2'-O-(2-methoxy)ethyl-gapmer backbone (OGX-011), and the optimal dose of OGX-011 capable of inducing </= 90% suppression of clusterin in human prostate cancer tissue was determined. Collectively, these findings suggest the utility of inactivating clusterin function using AS ODN technology as a novel therapeutic strategy for prostate cancer treatment. There have been four kinds of Phase II studies that have begun to further evaluate the efficacy of OGX-011 in patients with prostate, breast and lung cancers [202].

Clusterin protein was present in the cytoplasm of cervical cancer cells. The expression of clusterin protein in invasive cervical cancer tissues was not related to any clinicopathologic factors analyzed. Patients with positive clusterin expression showed significantly worse prognosis than those with negative clusterin expression ($p=0.017$). Multivariate analysis including clusterin expression revealed that clusterin expression ($p=0.006$) and the number of positive node groups ($p=0.002$) were independent prognostic factors for survival. Survival of patients with invasive cervical cancer could be stratified into

three groups by combination of clusterin expression and number of positive node groups with an estimated 5-year survival rate of 100.0% for no or one positive node group irrespective of clusterin expression (group A), 78.7% for multiple node groups with negative clusterin expression (group B), and 14.3% for multiple node groups with positive clusterin expression (group C) ($p=0.03$ for group A vs. group B, $p=0.004$ for group B vs. group C, and $p<0.0001$ for group A vs. group C). Clusterin expression and the number of positive node groups were independent prognostic factors for invasive cervical cancer patients treated with radical hysterectomy and systematic lymphadenectomy. Clusterin might be a new molecular marker to predict the survival of cervical cancer patients with multiple positive node groups [200].

Overexpression of the apoptosis-related protein clusterin is associated with breast cancer development and tumor progression. Clusterin-specific antisense oligonucleotides and antibodies were used to sensitize breast carcinoma cells to anticancer drugs routinely used in breast cancer therapy. MCF-7 and MDA-MB-231 cells were treated with the oligonucleotide or antibody, chemotherapeutic agents (doxorubicin or paclitaxel), tamoxifen, or with combinations of these. Treatments that include antisense clusterin oligonucleotide or antibody to clusterin have been shown to reduce the number of viable cells more effectively than treatment with the drugs alone. It was also demonstrated that dexamethasone pretreatment of breast cancer cell lines inhibits chemotherapy-induced cytotoxicity and is associated with the transcriptional induction of clusterin. However, anticlusterin treatment increases chemotherapy-induced cytotoxicity, even in the presence of glucocorticoids, suggesting a possible role for these proteins in glucocorticoid-mediated survival. These data suggest that combined treatment with antibodies to clusterin or antisense clusterin oligodeoxynucleotides and paclitaxel, doxorubicin, or tamoxifen could be a novel and attractive strategy to inhibit the progression of breast carcinoma by regulation of the clusterin function. Moreover, glucocorticoid activation in breast cancer cells regulates survival signaling by the direct transactivation of genes like clusterin which encode proteins that decrease susceptibility to apoptosis. Given the widespread clinical administration of dexamethasone before chemotherapy, understanding glucocorticoid-induced survival mechanisms is essential for achieving optimal therapeutic responses [201].

KIDNEY

Age

Because renal function decreases with advancing age in the obese Zucker rat, clusterin mRNA expression was examined in the kidney of young adult Zucker rats and compared with age-related changes in renal clusterin mRNA expression in Fischer 344 (F344) rats. Renal clusterin mRNA levels in the obese Zucker rat were 2.5-fold higher by 3 mo of age and fourfold higher at 5 mo of age compared with the lean strain. In comparison, renal clusterin mRNA in 12-mo-old F344 rats was twofold higher than in 3-mo-old animals and was tenfold higher at 24 mo of age. Clusterin mRNA was positively correlated with urinary protein excretion and negatively correlated with creatinine clearance in Zucker rats. Clusterin was increased in select nephrons of the obese Zucker rat kidney and in 24-mo-old F344 rat kidney as assessed by in situ hybridization. Increased expression of clusterin mRNA occurred mostly in the tubular epithelium of dilated, convoluted proximal tubules. These data indicate that renal clusterin mRNA levels increase as a function of age and that age-related increases in renal clusterin and the associated tubular abnormalities are accelerated in obese Zucker rats [89].

Aging mice deficient in apoJ/clusterin developed a progressive glomerulopathy characterized by the deposition of immune complexes in the mesangium. Up to 75% of glomeruli in apoJ/clusterin-deficient mice exhibited moderate to severe mesangial lesions by 21 months of age. Wild-type and hemizygous mice exhibited little or no glomerular pathology. In the apoJ/clusterin-deficient mice, immune complexes of immunoglobulin G (IgG), IgM, IgA, and in some cases C1q, C3, and C9 were detectable as early as 4 weeks of age. Electron microscopy revealed the accumulation of electron-dense material in the mesangial matrix and age-dependent formation of intramesangial tubulo-fibrillary structures. Even the most extensively damaged glomeruli showed no evidence of inflammation or necrosis. In young apoJ/clusterin-deficient animals, the development of immune complex lesions was accelerated by unilateral nephrectomy-induced hyperfiltration. Injected immune complexes localized to the mesangium of apoJ/clusterin-deficient but not wild-type mice. These results establish a protective role of apoJ/clusterin against chronic glomerular kidney disease and support the hypothesis that apoJ/clusterin modifies immune complex metabolism and disposal [142].

Unilateral Uretheral Obstruction (UUO)

The onset of hydronephrosis following unilateral ureteral obstruction is associated with the induced expression of clusterin RNA and protein. Within 30 minutes of obstruction, clusterin mRNA expression was localized to the adventitial layers of the hilar arteries and intrarenal arterioles. Increasing time of obstruction resulted in the notable absence or depletion of this layer. In addition, the pattern of clusterin expression changed with time to the collecting ducts and distal tubules. This study identifies the vascular support tissue of the kidney as the initial site of reaction and potential cell death following ureteral obstruction. This observation may be of importance in explaining the early alterations in blood flow associated with hydronephrosis [7].

With unilateral ureteral obstruction in rabbits as the experimental model, the time course of alterations in renal function, clusterin mRNA accumulation, and concentrations of clusterin protein in serum, urine, and renal tissue were investigated. RBF, GFR, and renal concentrating ability (percent sodium reabsorption and urine osmolarity) all decreased ($P < 0.05$) in the obstructed kidney from control values within 1 day of ureteral obstruction. Clusterin mRNA levels started to rise in the ipsilateral kidney within 12 h of ureteral obstruction and increased up to 10-fold above control levels after 3 days of obstruction. Hybridization histochemistry showed that clusterin mRNA was initially detectable in collecting ducts and distal tubules within 12 h of ureteral obstruction. After 7 days of obstruction, increased accumulation of clusterin mRNA was also detectable in proximal tubular epithelial cells. Clusterin gene expression remained elevated in collecting ducts after 60 days of obstruction. Clusterin expression in the contralateral kidney was increased twofold over control values after 12 h of obstruction. No increase in clusterin mRNA accumulation was detectable after 24 h in the contralateral kidney. Total clusterin protein in the obstructed kidney increased from 0.59 +/- 0.66 (mean +/- 1 SD) to 2.5 +/- 1.3 micrograms after 7 days of ureteral obstruction ($P < 0.05$) [32].

Compared with the adult, UUO in the neonate induces greater apoptosis, which in turn contributes to reduced renal DNA. This may be modulated by relative suppression of clusterin in the obstructed neonatal kidney due to greater activation of the renin-angiotensin system [70].

Real-time RT-PCR revealed an immediate increase in the clusterin mRNA level in the kidney, within 6 hours after UUO, and also maintenance of the mRNA expression level from day-1 to day-3 was 60-fold higher in the UUO kidney than in the sham kidney. ISH analysis revealed clusterin mRNA signals in the UUO renal tubular epithelium, whereas no signal was observed in the sham kidney. Detection of clusterin-alpha and -beta was conducted using the subtype-specific antibodies, by both of western blotting and immunohistochemistry. Although clusterin-alpha was predominant in the UUO urine, only faint signals were noted at the brush border of the tubular epithelium or intraductal. On the other hand, strong signals of clusterin-beta were detected in the UUO kidney homogenate, and the molecule was localized in the renal tubular epithelium. These results suggest that clusterin was translated in the renal tubular epithelium after de novo expression induced by renal injury. Thus, detection of clusterin mRNA and clusterin-beta in the kidney or clusterin-alpha in the urine may be useful for predicting nephrotoxicity [182].

In a subsequent study, animals were subjected to unilateral ureteral obstruction (UUO) in the first 2 days of life, and renal TGF-beta1 and clusterin mRNA were measured 3 days later. Rats were divided into treatment groups receiving saline vehicle, ANG, losartan (AT(1) receptor inhibitor), or PD-123319 (AT(2) receptor inhibitor). ANG stimulated renal TGF-beta1 expression via AT(1) receptors, a response similar to that in the adult. In contrast, clusterin expression was stimulated via AT(2) receptors, a response differing from that in the adult, in which ANG inhibits clusterin expression via AT(1) receptors. It was speculated that the unique response of the neonatal hydronephrotic kidney to ANG II is due to the preponderance of AT(2) receptors in the developing kidney [118].

Losartan increased clusterin expression by 60% in obstructed kidneys and seven-fold in intact kidneys in the unilateral ureteral obstruction model [54].

Pressure

Simulated glomerular hypertension increased mRNA expression of clusterin (0.55 +/- 0.05 vs 1.08 +/- 0.12, $P < 0.01$, $n = 3$) in mesangial cells when compared to cells grown under simulated normal glomerular pressure [95].

Oxidative injury

A single cell suspension of LLC-PK1 cells (porcine proximal tubular cell line) treated with albumin (control) was compared to cells aggregated with fibrinogen or purified human clusterin (aggregation graded 0 to 4). Following aggregation, the cells were injured with 1.5 mM hydrogen peroxide (H2O2) for three hours. Cell aggregation induced by clusterin but not fibrinogen pro-

tected against oxidant injury by H2O2. Complete abrogation of cytotoxicity occurred at a clusterin concentration of 2.5 micrograms/ml, which resulted in an aggregation score of 1. In the absence of aggregation, clusterin at concentrations of 20 and 50 micrograms/ml, but not lower doses, partially protected against injury induced by H2O2. Cell aggregation induced by both clusterin and fibrinogen partially protected against endogenously generated oxidant stress induced by incubating LLC-PK1 cells with aminotriazole and 1-chloro-2,4-dinitrobenzene (CDNB). In conclusion, clusterin protects against models of oxidant stress in vitro, whether generated by exogenously administered hydrogen peroxide, or from endogenously produced peroxide, and such protective effects can accrue from aggregative and nonaggregative properties of clusterin [98].

Glomerulonephritis

Using an antibody to rat clusterin as an immunofluorescent probe, clusterin deposits were demonstrated in the passive Heymann nephritis model along the glomerular capillary wall in an identical pattern to rat C3 and C5b-9. Decomplementation using cobra venom factor prevented proteinuria and intraglomerular MAC formation. The epimembranous clusterin were not detected in the complement-depleted animals. The role of clusterin in the mediation of glomerular injury remains unknown, but it is probably related to in situ formation of the terminal complement cascade where it may play a regulatory role [8].

Using the complement-dependent isolated perfused rat kidney model of autologous phase passive Heymann nephritis, the effect of clusterin depletion of perfused plasma on the development of glomerular injury was studied. Kidneys with planted glomerular sheep anti-rat Fx1A antibody were perfused with human plasma either depleted of clusterin to < or = 30%, or control plasma depleted of plasma fibronectin. Glomerular injury was then initiated by the addition of guinea pig anti-sheep immunoglobulins to the perfusate. Kidneys perfused with clusterin depleted plasma developed significantly greater proteinuria at all time points when compared to control kidneys. Glomerular antibody binding and C3 deposition were similar in the two groups, but terminal complement components were deposited in larger amounts in the clusterin depleted group. These data support a possible role for clusterin in vivo in the protection of complement-induced glomerular injury [43].

Renal biopsies of 60 membranous glomerulonephritis (MGN) patients by immunohistochemistry utilizing antibodies against clusterin, C5b-9, and phosphorylated-protien kinase C (PKC) isoforms (pPKC) were studied. In vitro experiments were performed to investigate the role of clusterin during podocyte damage by MGN serum and define clusterin binding to human podocytes, where megalin is known to be absent. Clusterin, C5b-9, and pPKC-alpha/beta showed highly variable glomerular staining, where high clusterin profiles were inversely correlated to C5b-9 and PKC-alpha/beta expression (P=0.029), and co-localized with the low-density lipoprotein receptor (LDL-R). Glomerular clusterin emerged as the single factor influencing proteinuria at multivariate analysis and was associated with a reduction of proteinuria after a follow-up of 1.5 years (-88.1%, P=0.027). Incubation of podocytes with MGN sera determined strong upregulation of pPKC-alpha/beta that was reverted by pre-incubation with clusterin, serum de-complementation, or protein-A treatment. Preliminary in vitro experiments showed podocyte binding of biotinilated clusterin, co-localization with LDL-R and specific binding inhibition with anti-LDL-R antibodies and with specific ligands. These data suggest a central role for glomerular clusterin in MGN as a modulator of inflammation that potentially influences the clinical outcome. Binding of clusterin to the LDL-R might offer an interpretative key for the pathogenesis of MGN in humans [174].

Increased expression of clusterin in cultured rat glomerular mesangial cells stimulated by sublytic complement attack was observed. Clusterin was induced in glomerular mesangial cells during the course of immune-mediated injuries. This up-regulation of clusterin may play a critical role in protecting mesangial cells from complement attack [127].

A study was designed to define the sites of clusterin mRNA accumulation in murine lupus-like nephritis in comparison with murine tubulopathies. In lupus-like nephritis, a significant increase of clusterin mRNA abundance was demonstrated. This up-regulation was localized exclusively in tubular epithelial cells exhibiting tubulointerstitial alterations, whereas no clusterin mRNA was detectable in diseased glomeruli, excluding an active synthesis of clusterin by glomerular cells. A similar tubular increase of clusterin mRNA abundance was observed in myeloma-like cast nephropathy induced by IgG3 monoclonal cryoglobulins and even in the absence of any detectable histological alterations in a model of septic shock induced by the injection of bacterial lipopolysaccharides. These results suggest that tubular epithelial cells are

the only sites of clusterin mRNA accumulation during the course of lupus-like nephritis and that the tubular up-regulation of clusterin gene expression may reflect the cellular response to various types of tubular injuries [93].

Proteinuria-induced renal disease

We studied proteinuria-induced renal disease and its influence on clusterin-mediated apoptosis. Exposure of cultured mouse proximal tubule epithelial cells to bovine serum albumin (BSA) resulted in activation of NF-kappaB and activator protein-1 (AP-1) within hours followed by a decline in their activation, decreased activation of extracellular signal-regulated kinases (ERK1/2), decreased cell-associated antiapoptotic Bcl-xL protein but increased apoptosis. Clusterin progressively increased in the media over a 3 day period. Clusterin siRNA blocked protein production, increased NF-kappaB activation, and significantly increased cellular Bcl-xL protein, thereby reducing spontaneous and BSA-induced apoptosis. An siRNA to the NF-kappaB inhibitor IkappaBalpha had similar results. BSA-stimulated NF-kappaB activation reciprocally decreased AP-1 activity by preventing ERK1/2 phosphorylation. These in vitro studies suggest that clusterin inhibits NF-kappaB-mediated antiapoptotic effects by the apparent stabilization of IkappaBalpha switching from promoting inflammation to apoptosis during proteinuria [189].

On the other hand, the induction of clusterin after folic acid administration or subtotal nephrectomy was independent of the presence of an intact complement system, because similar increases in clusterin expression were observed in C5-sufficient and C5-deficient mice [33].

Renal Ischemia

Clusterin gene expression was greatly increased 24 to 72 hours after experimental renal ischemia and began decreasing at 96 hours. This selective sequence of gene expression or repression after renal ischemia might maximize the proliferative repair process [9].

Clusterin mRNA found to be highly expressed in the 30-minute arterial clamped rat kidney after 24 hours of reperfusion, but was not detectable in mRNA extracted from renal tissue after 24 hours chronic infarction. This demonstrates that brief periods of complete ischemia initiate a form of cell death (apoptosis) during a subsequent reperfusion phase that is drastically different from cellular necrosis induced by prolonged severe ischemia [21].

The effect of the angiotensin-converting enzyme inhibitor captopril on clusterin mRNA was examined in partially nephrectomized male rats. Urine protein excretion was measured 3, 7, and 28 days after removal of five sixths of the renal mass. Nephrectomy caused a progressive increase in clusterin mRNA levels in the remnant kidney. Maximal clusterin mRNA levels occurred 7 days after nephrectomy and declined 28 days after nephrectomy. Captopril, 250 mg/ml in drinking water, prevented the injury-induced increase in clusterin mRNA at 7 and 28 days. Captopril had no effect on clusterin in sham-operated rats. As expected, the urine protein excretion increased progressively after nephrectomy, and this was attenuated by administration of captopril in the drinking water. Therefore, clusterin is a marker of renal injury which, along with proteinuria, is modulated by angiotensin-converting enzyme inhibition [102].

Persistently increased clusterin mRNA and protein was seen in the peri-infarct zone following 1-1/3 nephrectomy. This increased expression of clusterin may be playing a role in the ischemia-related apoptosis present in the scar-adjacent tissue [18].

Polycystic Kidney Disease

The expression of the clusterin gene in polycystic kidneys of the C57BL/6J-cpk mouse was investigated, a model of autosomal recessive polycystic kidney disease in which there is development of epithelial-lined cysts arising primarily from the collecting duct system. Abnormally high levels of clusterin mRNA were found in the cyst wall epithelium of polycystic kidneys. The expression of the clusterin gene in normal development suggests that it plays a role in differentiating epithelial structures; and the abnormally high levels of clusterin gene expression in polycystic kidneys suggests that the cells lining cysts are not fully differentiated. It is possible, therefore, that polycystic kidney disease is caused by a defective developmental process in which there is a delay in terminal differentiation [12].

In autosomal dominant polycystic kidney disease (ADPKD) versus normal kidney (NK) cell monolayers, synthesis of sulfated glycoproteins is impaired, processing of sulfated glycoproteins by the Golgi apparatus is prolonged, and assembly of these macromolecules into the extracellular matrix is reduced. These alterations may have a fundamental role in the pathogenesis of autosomal dominant polycystic kidney disease [35].

Clusterin immunostaining was examined in nephrectomy specimens from patients with autosomal-dominant polycystic kidney disease (N = 5), autosomal-recessive polycystic kidney disease (N = 3), multilocular cyst of the kidney (N = 2), renal hypoplasia/dysplasia (N = 7), Wilms' tumor (nephroblastoma) (N = 6), renal cell carcinoma (N = 9), and acute and/or chronic renal transplant rejection (N = 15). No clusterin staining was detected in normal renal tissue distant from renal cell carcinomas. Increased expression of clusterin was found in epithelial cells lining cysts in all of the cystic disorders studied. Clusterin expression was found in some immature tubules in hypoplastic/dysplastic kidneys and in tubules of rejected renal allografts, but was not a prominent finding in renal neoplasms, although some renal cell carcinomas expressed clusterin in a focal manner. Common features of clusterin induction included exclusively epithelial production of clusterin in cysts, immature nephrons, and injured tubules, heterogeneity of clusterin expression, with only some tubules and/or cysts in a given area staining for clusterin, and uniform clusterin staining of epithelial cells in a given tubule or cyst in most cases. Based on its cohesive properties, it was speculated that clusterin functions to maintain cell-cell and cell-substratum interactions which become perturbed in the setting of renal injury and cystic diseases [44].

Phenol II is a cystogenic chemical that rapidly induces renal cysts, which regress after drug withdrawal. Cyst formation in this model parallels changes in the tubular basement membrane. Clusterin is a potent cohesive factor induced in states of tissue remodeling. The purpose of this study was to determine if renal clusterin was increased in the Phenol II model and to define the time course and distribution of its induction. Male Sprague-Dawley rats were given, by daily gavage, Phenol II (1.2 mg/kg per day) or vehicle (control). The kidneys were harvested after 1, 2, or 4 days of Phenol II treatment or 3 or 7 days after drug withdrawal. An increase in immunoreactive clusterin was seen in the kidneys of Phenol II-treated rats but not in controls. The appearance of clusterin followed a time course similar to that for cyst formation, with expression confined to the epithelial lining and intratubular casts of dilated or cystic tubules. After Phenol II withdrawal, renal cysts regressed and clusterin staining disappeared. The development of cysts was associated with an increase in clusterin mRNA that decreased after drug withdrawal. In conclusion, a marked, yet reversible induction of clusterin occurred in chemically induced polycystic kidney disease [52].

RENAL TOXICITY

Gentamycin

Adult Lewis rats were treated with 100 mg/kg/day of gentamicin sulfate for 12 days. Urine, serum, and tissue levels of clusterin protein were measured, as were urinary N-acetyl beta-glucosaminidase (NAG) and serum creatinine levels. Induction of renal injury by gentamicin was detectable within 4 days by increased levels of urinary N-acetyl beta-glucosaminidase (from 280 +/- 66 (mean +/- SD) to 910 +/- 210 nmol/mg creatinine), and within 9 days of initiating gentamicin treatment by increased serum creatinine (from 0.5 +/- 0.1 to 1.2 +/- 0.4 mg/dl). Paralleling these changes, renal, urinary, and serum levels of clusterin increased 10-, 116-, and 3-fold (P less than 0.05). Treatment with gentamicin sulfate did not increase clusterin levels in the seminal vesicle, ventral prostate, testis, or epididymis. The measurement of urinary or serum clusterin may play a role in the early detection of renal injury [15].

LLC-PK(1) cells were incubated with varying concentrations of gentamicin in serum-free media, and cytotoxicity was quantified by lactate dehydrogenase release and confirmed by vital dye exclusion. A dose-dependent increase in cytotoxicity occurred with gentamicin concentrations up to 27 mg/ml. Clusterin decreased cytotoxicity in a dose- and time-dependent manner at 6, 12, and 24 h, whereas albumin, used as a control protein, had no effect. In contrast to the aminoglycoside model, when cells were injured by depletion of ATP, clusterin had only a minimally protective effect. LLC-PK(1) cells did not express megalin, a receptor that can mediate the uptake of both clusterin and aminoglycosides into proximal tubule cells. Uptake of gentamicin into LLC-PK(1) cells was observed despite the absence of megalin. In conclusion, clusterin specifically protects against gentamicin-induced renal tubular cell injury by a megalin-independent mechanism [143].

Cyclosporine A

A study examined the expression of the clusterin gene in the kidney during the development of cyclosporine (CyA)-induced nephrotoxicity in the rat using in situ hybridization histochemistry. Female Sprague-Dawley rats (170 g) were divided into experimental or control groups and were given intraperitoneal injections of CyA (25 mg/kg) or vehicle respectively for 2, 4 and 6 weeks. Kidneys from animals sacrificed at these times were fixed in 4% paraformaldehyde and embedded in paraffin. Tissue sections were hybridized using either a sense or antisense riboprobe complementary to clusterin mRNA

which was labelled with 32P-UTP. Clusterin gene expression was detected in scattered tubules in kidneys from control animals. Expression of clusterin in cortical collecting ducts of CyA-treated animals was evident at 2 weeks and increased substantially at 4 and 6 weeks. Clusterin expression was also seen in afferent arterioles, the glomerular capsule and transitional epithelium of the renal pelvis of kidney sections from rats treated with CyA for 6 weeks. No labelling above background was seen at any time with the sense probe. Renin immunostaining in afferent arterioles of kidney sections from animals treated with CyA showed a marked increase after 4 and 6 weeks of CyA treatment [58].

Puromycin

Male Wistar rats (weighing 251 +/- 16 g) were treated with puromycin aminonucleoside (PAN: 15 mg/100 g body wt, subcutaneously; n = 7) or vehicle (control; n = 8). The kidneys were harvested 6 d after treatment, when rats were nephrotic. Clusterin mRNA was markedly induced in the kidneys of nephrotic rats (8.5-fold versus control). Immunohistochemistry studies demonstrated clusterin primarily in tubules in the cortex and medulla. Many of the tubules staining for clusterin were dilated but had no other differentiating morphologic features. Increased numbers of proliferating tubular cells were seen at 6 d, but there was no correlation between these cells and clusterin staining. In contrast to the extent and pattern of clusterin staining, vimentin was seen in only sporadic, dilated tubules, in addition to its expected glomerular localization. An increase in clusterin mRNA was not seen 1, 2, or 4 d after PAN injection. In conclusion, tubular epithelial cell induction of clusterin occurs in the kidneys of nephrotic rats. The appearance of clusterin precedes the development of tubulointerstitial disease and may be a response to the proteinuria [88].

Cisplatin

Sprague-Dawley rats were treated with intravenous cisplatin (6 mg/kg) or vehicle. Serum creatinine concentrations were measured and kidneys harvested at 1, 2, and 5 days. Marked induction of clusterin mRNA was seen only at 5 days, a time when serum creatinine concentration was the highest. Histology of kidney tissue 5 days after cisplatin administration revealed marked tubular necrosis localized to the outer stripe of the outer medulla, a region rich in proximal tubules. Immunohistochemistry and in situ hybridization at 5 days demonstrated clusterin primarily in the inner stripe of the outer medulla. In conclusion, expression of clusterin follows renal injury with cisplatin at a time corresponding to the morphologic evidence of tubular necrosis and cell detachment; quite surprisingly, such expression occurs at a site distant from the primary injury [76].

Glycerol

In glycerol-induced acute renal failure, a model of rhabdomyolysis, clusterin mRNA was markedly increased 24 hours after injection of glycerol (control 97 +/- 21 versus glycerol 3644 +/- 134 optical density units; $p < 0.001$). Immunohistochemical clusterin was also increased in glycerol-treated rats with tubules in both cortex and medulla staining for clusterin. In vitamin E and selenium deficiency, clusterin mRNA was increased 9 weeks after initiation of the deficient diet (control 97 +/- 13 versus deficient 1137 +/- 403 optical density units; $p < 0.04$) as were the number of tubules staining for clusterin. Since renal injury is instigated in the glycerol model by muscle damage, we tested the effect of muscle extract on clusterin expression in vitro. A homogenate of skeletal muscle induced clusterin mRNA and this induction was not associated with disruption of cell membranes and was not inhibited by cycloheximide treatment, but was blocked by actinomycin D. Since increased generation of hydrogen peroxide is a pivotal biochemical lesion in both in vivo models, we tested the effect of peroxide to induce clusterin in vitro; no such induction occurred. Thus, renal tubular clusterin expression was increased in both acute glycerol-induced renal failure and chronic vitamin E and selenium deficiency, two in vivo models of oxidant injury to the kidney. In vitro induction of clusterin can occur and can be dissociated from cell injury [50].

Nefiracetam

The occurrence of renal papillary necrosis (RPN), seen only in dogs after repeated oral administration of nefiracetam, a neurotransmission enhancer, at a relatively high dose, is because of inhibition of renal prostaglandin synthesis by the nefiracetam metabolite M-18. By Western blot and quantitative real-time RT-PCR analysis with renal morphological aspects, individual findings showed that renal clusterin mRNA was increased in dogs with severe renal injury. Changes in renal clusterin mRNA may reflect the progression or severity of RPN [167].

In a study, a single cell suspension of renal epithelial (LLC-PK1) cells was treated with purified human clusterin, resulting in time- and dose-dependent cell aggregation. Electron microscopy of the cell aggregates demonstrated cell junction and lumen formation. To determine the effect of clusterin on cell

adhesion, tissue culture plates were coated with clusterin, fibronectin, PBS, or albumin. Clusterin and fibronectin promoted cell adhesion to the same extent. The adhesion to clusterin was dose dependent and specific, as a monoclonal antibody against clusterin inhibited cell adhesion to clusterin but not fibronectin. Perterbations of the cytoskeleton may underlie the alterations in cell attachment which occur in renal injury. Induction of clusterin mRNA was seen after disruption of both microtubules and microfilaments and after inhibition of cell-substratum interactions. In conclusion, clusterin is a potent renal epithelial cell aggregation and adhesion molecule. It is speculated that clusterin functions to promote cell-cell and cell-substratum interactions which are perturbed in the setting of renal injury, thereby preserving the integrity of the renal epithelial barrier [60].

HEART

In the normal heart, abundant clusterin mRNA and protein are expressed in atrial myocytes; no expression is detected in ventricular myocytes. To provide clues about the role of clusterin in the heart, the response of clusterin to heart disease, including three models of myocarditis and two models of in vivo pressure overload hypertrophy, were examined. In the disease model studied extensively, myosin-induced myocarditis, in situ hybridization detected induction of clusterin mRNA in ventricular myocytes immediately before histological evidence of injury. Clusterin message in ventricular myocytes reached high levels as myocarditis became more severe. Evidence of early clusterin induction, before inflammation and injury, also occurred in viral myocarditis. Clusterin mRNA was not present in the inflammatory or interstitial cells during myocarditis. In areas of severe inflammation and myocardial fiber degeneration, clusterin showed a gradient of expression, with highest levels in myocytes immediately surrounding the lesion and diminishing with increasing distance. Clusterin protein also accumulated in myocytes at the interface between degenerated myocardial tissue and the surrounding cardiac tissue. During cardiac hypertrophy that occurred without associated inflammation or cell damage, ventricular clusterin mRNA was not detected. When ischemic damage accompanied hypertrophy, clusterin was induced in the ventricular myocytes near the lesion borders. The correlation of clusterin induction with ventricular tissue damage, but not hypertrophy, suggests that clusterin is a repair response protein. It is proposed that clusterin functions to limit tissue injury and/or promote tissue remodeling [66].

Clusterin-deficient and wild-type mice exhibited similar initial onset of myocarditis, as evidenced by the induction of two early markers of the T cell-mediated immune response, MHC-II and TNF receptor p55. Furthermore, autoantibodies against the primary antigen cardiac myosin were induced to the same extent. Although the same proportion of challenged animals exhibited some degree of inflammatory infiltrate, inflammation was more severe in clusterin-deficient animals. Inflammatory lesions were more diffuse and extensive in clusterin-deficient mice, particularly in females. In marked contrast to wild-type animals, the development of a strong generalized secondary response against cardiac antigens in clusterin-deficient mice was predictive of severe myocarditis. Wild-type mice with a strong Ab response to secondary antigens appeared to be protected from severe inflammation. After resolution of inflammation, clusterin-deficient, but not wild-type, mice exhibited cardiac function impairment and severe myocardial scarring. These results suggest that clusterin limits progression of autoimmune myocarditis and protects the heart from postinflammatory tissue destruction [124].

Clusterin is selectively deposited in the infarcted areas of human myocardium. Clusterin deposits were observed in the heart tissue of 10 patients whose infarcted lesions were 8 hr to 14 days old, but not in patients who died from other causes. Clusterin co-localized with the komplement membrsne attack complex on the surface of damaged cardiomyocytes. In normal myocardium, only endothelial lining of blood vessels occasionally stained positive for clusterin. The 80,000 MW clusterin was also detected by Western blot analysis in extracts of myocardial infarction lesions, but only faintly in extracts of normal heart. As clusterin has apparently failed in protecting myocardium against complement-mediated cell injury its main role might be to participate in the clearance of damaged and necrotic tissue [38].

Sprague-Dawley rats underwent permanent coronary ligation or sham operation. Hearts were harvested at 6 hours and at 2, 14, and 28 days after infarction. Cardiac clusterin expression was examined by immunohistochemistry and in situ hybridization. Left ventricular clusterin staining was evident at 6 hours and 2 days after myocardial infarction, although not at later time periods. Clusterin was localized to peri-infarct zone myocytes and endothelial cells of this region, and local synthesis of clusterin by myocytes was confirmed by in situ hybridization. Clusterin was not present in inflammatory cells or in left ventricular tissue distant from the infarct. The distribution of clusterin was different from the membrane attack complex of complement (C5b-9),

with the latter being present diffusely throughout the infarct zone. Although the role of cardiac clusterin is not known, we speculate that clusterin's cohesive properties serve to promote myocyte interactions that are perturbed in the peri-infarct zone after myocardial infarction [91].

The relationship between clusterin and activated complement in human heart infarction was examined and the effect of this protein on ischemic rat neonatal cardiomyoblasts (H9c2) and isolated adult ventricular rat cardiomyocytes as in vitro models of acute myocardial infarction was evaluated. Clusterin protects cells by inhibiting complement and colocalizes with complement on jeopardized human cardiomyocytes after infarction. The distribution of clusterin and complement factor C3d was evaluated in the infarcted human heart. The protein expression of clusterin in ischemic H9c2 cells was also analyzed. The binding of endogenous and purified human clusterin on H9c2 cells was analyzed by flow cytometry. Furthermore, the effect of clusterin on the viability of ischemically challenged H9c2 cells and isolated adult ventricular rat cardiomyocytes was analyzed. In human myocardial infarcts, clusterin was found on scattered, morphologically viable cardiomyocytes within the infarcted area that were negative for complement. In H9c2 cells, clusterin was rapidly expressed after ischemia. Its expression was reduced after reperfusion. Clusterin bound to single annexin V-positive or annexin V and propidium iodide-positive H9c2 cells. Clusterin inhibited ischemia-induced death in H9c2 cells as well as in isolated adult ventricular rat cardiomyocytes in the absence of complement. We conclude that ischemia induces the upregulation of clusterin in ischemically challenged, but viable, cardiomyocytes. These data suggest that clusterin protects cardiomyocytes against ischemic cell death via a complement-independent pathway [166].

EtOH-exposed canine fetal myoblasts underwent apoptosis in a concentration- and time-dependent manner. Expression of clusterin by cDNA transfection markedly reduced EtOH-induced apoptosis in the cells. Clusterin expression also restored partially the mitochondrial membrane potential and prevented the release of cytochrome-c from mitochondria into cytoplasma. Thus, clusterin serves as a cytoprotective protein that protects cardiac stem cells against EtOH cytotoxicity [180].

LIVER

A sudy determined the kinetics of clusterin expression at different post-transplantation time points in a tolerogenic model (DA-PVG) where rejection was naturally overcome without any immunosuppressive drugs in comparison with the syngenic orthotopic liver transplantation (OLT) model (DA-DA). Peripheral blood and liver tissues were taken from OLT at various post-operative time points. A strong expression of soluble clusterin was observed on post-transplantation day 7, which occurred at the peak of the rejection in this tolerogenic OLT model. The expression of clusterin remained strong even after tolerance was achieved. The intensity of clusterin expression was much stronger when compared with the syngenic OLT (DA-DA) model after OLT. A strong expression of clusterin mRNA was also observed in the tolerogenic model on post-OLT day (POD) 7 and the expression persisted when compared with the syngenic model on post-OLT day 60. These data have shown that the strongest levels of clusterin during the reaction phase in tolerogenic OLT may be involved in tolerance induction [122].

NEURAL TISSUE

Studies have demonstrated the overexpression of clusterin in various CNS disorders, such as certain gliomas, Alzheimer's disease and epilepsy, as well as after experimental brain injury in animals where different cell types were undergoing tissue remodelling or cell death [197].

Clusterin is a ubiquitous multifunctional glycoprotein with the capability to interact with a broad spectrum of molecules, among them the Alzheimer's Abeta peptide. Due to its co-localization with fibrillar deposits in systemic and cerebral amyloid disorders, clusterin is also considered an amyloid-associated protein. Although no genuine function has been attributed to this protein so far, it has been implicated in a wide variety of physiological and pathological processes, a role that may vary according to the protein maturation, sub-cellular localization, and the presence of certain tissue- or cell-specific factors [203].

The abundance of clusterin RNA is increased in hippocampus from patients with Alzheimer disease and Pick disease [194].

Ibotenic acid lesioning that caused death of striatal neurons also stimulated astrocytic responses as monitored by GFAP and clusterin [195].

One clone was noticeably increased in retinitis pigmentosa in comparison with the control: partial sequencing showed it encoded clusterin [196].

Adult male Wistar rats were subjected to 30 min of forebrain ischemia by four vessel occlusion. By 3 days after the ischemic insult, clusterin RNA levels were increased two fold in caudate nucleus and hippocampus. Clusterin protein levels assessed by immunoblots were markedly increased in both brain

regions following ischemia. Glial fibrilary acidit protein (GFAP) RNA levels also increased over 5 fold in caudate nucleus and hippocampus following the ischemic insult. Despite significant elevations in GFAP RNA, protein levels of GFAP assessed by immunoblot were only marginally affected. The elevated expression of clusterin in rodent brain following this and other experimental lesion paradigms (e.g., excitotoxic lesions, deafferentation) suggest some general involvement of clusterin in neurodegeneration and remodelling following neuronal injury [198].

SEMINIFEROUS SYSTEM AND SPERMATOZOA

Clusterin is one of the major proteins secreted by rat Sertoli cells and epididymal cells in culture. The disulfide-linked dimeric protein secreted by Sertoli cells and found in seminiferous tubule fluid is composed of monomers of Mr 47 000 and 34 000 whereas the epididymal protein exhibits monomers of Mr 40 000 and 29 000. When both forms were chemically or enzymatically deglycosylated, they yielded proteins of similar molecular weight. No modification of the higher molecular weight testicular form by epididymal cells or fluids could be detected in incubation media. Clusterin mRNA was localized in epididymal epithelium by in situ hybridization. Northern blot analysis indicated the testicular and epididymal mRNAs were of similar size. These findings suggest that the two forms of the protein occur because of tissue-specific post-translational modifications. The detergent-extracted protein from washed testicular spermatozoa is of the higher molecular weight form while epididymal sperm carry the lower molecular weight form. Immunohistochemical evidence suggests that the testicular form is removed prior to the initial segment of the epididymis and the epididymal form is applied in the proximal caput epididymidis. Clusterin was immunolocalized to the sperm membrane at the ultrastructural level and was distinctly different from the immunolocalization of outer dense fiber proteins and fibrous sheath proteins [11].

Later studies confirmed thatt two forms of clusterin, the beta-chain monomer and the heterodimer, are present on the spermatozoal surface membrane and in seminal plasma [103].

Transfection of ASC-17D Sertoli cells with a 20-base antisense oligonucleotide against clusterin mRNA resulted in extracellular release of LDH and DNA fragmentation. Sertoli cell death by antisense oligonucleotide of clusterin was sequence specific and dose dependent. Treatment of antisense oligonucleotide induced a marked reduction of synthesis for clusterin protein, but not for clusterin mRNA expression, suggesting the translational suppression of clusterin by antisense oligonucleotide. Further, microscopic observation showed that more noticeable cell death was induced by treating the antisense prior to plating the cells than by treating after cell attachment to the plate. From these results, we speculate that down-regulation of clusterin expression in the anchorage-dependent Sertoli cells prevents them from attaching to the plate, and therefore induces cell death [120].

Clusterin mRNA levels were measured by quantitative RT-PCR in RNA extracted from testicular biopsies of 49 azoospermic patients classified according to testicular histology as having normal spermatogenesis or spermatogenic failure. When related to the percentage of Sertoli cells counted on a histological section of a neighbouring tissue sample, clusterin mRNA levels were significantly lower in the ‚spermatogenic failure' group compared with the control group (P = 0.0043, respectively). These levels were also significantly lower in the cases of ‚constitutive' (cryptorchidism and Yq microdeletion) and ‚idiopathic' spermatogenic failures when compared with the control group; conversely, they were not significantly decreased in the group with ‚acquired spermatogenic failure' (orchitis, testicular traumatism, chemoradiotherapy and varicocele).These data further demonstrate an alteration of Sertoli cell functions in some human spermatogenic failures and suggest that a lack of Sertoli cell maturation may be involved in cases of constitutive or idiopathic spermatogenic failures [172].

Western blots of cauda sperm membrane extracts of control animals and orchiectomized animals treated with testosterone had a very low level of epididymal clusterin, whereas extracts collected from orchiectomized animals revealed high levels of clusterin. It was suggested that, in the normal animal, clusterin is secreted into the lumen of the proximal epididymis where it binds to the sperm membrane. In the distal epididymis, clusterin dissociates from sperm and is processed (proteolysis/endocytosis). I tis hypothesized that, in the absence of androgen, the processing and regulation of clusterin is disrupted [24].

It was proposed that protective effects of clusterin are the core of the differential tolerance of ischemia by the various testicular cells and the morpholo-

gy of the postischemic testis. Suppression of ischemic damage selectively in Sertoli cells might be the consequence of the ability to produce clusterin, an endogenous inhibitor of ischemic injury. This hypothetical function of clusterin is supported by its immunosuppressive properties and its structural and functional identity to several types of human complement cytolysis inhibitors [13].

In the initial segment and the caput epididymidis, neither orchidectomy nor testosterone replacement, at either dose, had any effect on clusterin mRNA concentrations. In the corpus and cauda epididymidis, bilateral orchidectomy resulted in a 3.5- and 9.4-fold increase, respectively, in clusterin mRNA concentrations, whereas testosterone replacement caused a dose-dependent decrease in clusterin mRNA concentrations. Unilateral orchidectomy was done to determine if clusterin mRNA concentrations are dependent on testicular factors released in the lumen of the epididymis. In the corpus and the cauda epididymidis, unilateral orchidectomy resulted in elevated clusterin mRNA concentrations in the ipsilateral epididymis. There were no changes in clusterin mRNA concentrations in the initial segment and caput epididymidis. These results provide complementary evidence that the message for clusterin is differentially regulated along the epididymis. During postnatal development clusterin mRNA concentrations in the caput-corpus epididymidis increased dramatically between 14 and 21 days as well as between 49 and 63 days. Interestingly, between 28 and 42 days, when serum testosterone concentrations are increasing, there was no change in the concentration of clusterin mRNA in the caput-corpus epididymidis. Similar results were observed in the cauda epididymidis with the exception that between 28 and 42 days, there was a dramatic decrease in clusterin mRNA in the cauda epididymidis. Together these experiments demonstrate that the regulation of clusterin mRNA concentrations is segment specific.In the initial segment and caput epididymidis there is no apparent regulation of clusterin by testicular factors,whereas in the corpus and cauda epididymidis testosterone can repress clusterin mRNA concentrations [19].

Morphologically abnormal spermatozoa have an extensive surface coating of conventional 80 kDa native clusterin, but this form of clusterin is not detectable on normal spermatozoa. Normal spermatozoa, however, contain within the acrosomal cap a different form of clusterin, reactive with an anticlusterin alpha-chain antibody. Agglutinated spermatozoa, most of which are grossly abnormal, were intensely labelled with the antibody against conventional 80 kDa clusterin, suggesting that the ‚clustering' properties of this protein may play a role in the aggregation of abnormal spermatozoa. Anticlusterin monoclonal antibodies may be useful for semen analysis. Staining spermatozoa with anticlusterin monoclonal antibodies is a technically simple method which provides a visually obvious means of assessing spermatozoa morphology and acrosome status simultaneously. The current data also suggest that different functions of clusterin in the reproductive tract may be attributed to different molecular forms of the protein [48].

The acrosomal form of clusterin is associated with the contents of the acrosome [129].

It was suggested that the percentage of clusterin positive cells (CPS) in bull semen is potentially a better predictor of fertility than sperm motility or abnormal morphology [125].

It was also suggested that the percentage of CPCs in ram semen could be a useful marker in poor-quality ejaculates [135].

It was demonstrated the expression of clusterin gene in four Leydig tumor cell lines, including mouse MA-10 and I-10 and rat R2C and LC-540. When the cells were incubated with 0.1 mM 8-bromo-cAMP or (Bu)2cAMP for 17 h, an unexpected, profound suppression of clusterin mRNA accumulation was observed. A 60-70% decrease in clusterin mRNA was observed in MA-10 and R2C cells, 10% in I-10 cells, and no apparent change in LC-540 cells. The inhibitory effect of cAMP was specific to the clusterin gene, since in the same cells cholesterol side-chain cleavage enzyme mRNA was drastically elevated in MA-10 and I-10 cells while alpha-tubulin mRNA levels were not changed in all four cell lines. The reduction could be detected as early as 4 h, and was evident at 17 h after cAMP administration. Removal of cAMP from culture media at 17 h prevented the decline of clusterin mRNA. The suppression of clusterin gene expression can also be demonstrated by treatment with human CG or forskolin, which were known to elevate intracellular cAMP levels22. The reduction of clusterin mRNA after exposure of MA-10 cells to cAMP is not due to a decrease in its transcriptional activity, but rather to an increase in the degradation of this mRNA through synthesis of a destabilizing protein(s) and its mRNA [66].

Follicle-stimulating hormone had no effect on clusterin in rat Sertoli cells [23].

UTERUS

In the mouse, clusterin mRNA was expressed in uterine luminal and glandular epithelial cells coincident with the presence of clusterin protein. The clusterin gene was differentially expressed in the glandular and uterine luminal epithelial cells during the estrous cycle and following hormone depletion. Expression of clusterin was not induced in ovariectomized mice by estrogen, progesterone, or dexamethasone treatment alone. Progesterone administration after an initial estrogen pretreatment, however, resulted in dramatic induction of clusterin as the progesterone level declined. In contrast, clusterin was not induced when a long-lived progesterone analog, medroxyprogesterone, was substituted for progesterone. In the human menstrual cycle, clusterin was present in glandular lumens only during the late secretory phase. Declining progesterone levels, causing substantial tissue reorganization, are characteristic of the times of marked clusterin induction in uterine epithelial cells. These expression patterns are consistent with clusterin functioning as an extracellular cytoprotectant by mediating clearance of and/or neutralizing cytolytic tissue debris [53].

Uterine clusterin gene activity was not detected immediately following fertilization, but glandular epithelial expression of clusterin mRNA appeared just before the time of blastocyst implantation and persisted postimplantation. During implantation, uterine luminal epithelial cells also expressed clusterin, but expression was excluded from luminal cells adjacent to the sites of attached blastocysts. clusterin protein accumulated in the glandular and uterine lumens in proximity to the epithelial cells that expressed clusterin mRNA. It was suggested that clusterin expression is a marker of uterine receptivity to blastocyst implantation. Subsequent expression of clusterin message in uterine stromal cell types and in circular muscle myocytes coincided with the onset of decidualization. During this period the myocytes of the longitudinal muscle layer showed no evidence of clusterin mRNA. Clusterin protein was localized to nondecidualized tissue but was not evident in decidualized cells. In contrast, the protein was dispersed throughout both the circular and longitudinal myometrium. In the uteri of hormone-treated females stimulated with oil, clusterin was also expressed during decidualization in stromal cells and in circular myocytes, indicating that signals specifically transmitted from the embryo itself are not responsible for clusterin mRNA accumulation [71].

Analysis of the expression of clusterin by quantitative reverse transcription-polymerase chain reaction detected an increase in the steady-state level of its transcripts in blastocysts from diabetic rats. In situ hybridization revealed that about half the cells identified as expressing clusterin mRNA exhibited signs of nuclear fragmentation. In vitro experiments demonstrated that high D-glucose increased nuclear fragmentation, TUNEL labeling and clusterin transcription. Tumor necrosis factor-alpha (TNF-alpha), a cytokine whose synthesis is up-regulated in the diabetic uterus, did not induce nuclear fragmentation nor clusterin expression but increased the incidence of TUNEL-positive nuclei. The data suggest that excessive cell death in the blastocyst, most probably resulting from the overstimulation of a basal suicidal program by such inducers as glucose and TNF-alpha, may be a contributing factor of the early embryopathy associated with maternal diabetes [87].

OVARY

A homogenous population of healthy and atretic follicles was obtained by treating immature rats with pregnant mare serum gonadotropin (PMSG). Apoptotic cell death was evaluated by TUNEL. Clusterin expression in the healthy and atretic follicles was examined by immunohistochemical and Western blot analyses, and gene expression was examined by Northern blot analysis. Clusterin protein and its mRNA are only expressed in granulosa cells of atretic follicles obtained from PMSG-treated rats on day 5 of the treatment. Healthy follicles from PMSG-treated rats on day 2 of the treatment do not express clusterin. Theca and stroma cells of both healthy and atretic follicles showed no signs of apoptosis and did not express clusterin. Withdrawal of trophic support from granulosa cells in cultures to induce apoptosis resulted in a dramatic increase in the levels of clusterin and its mRNA compared to cells cultured in serum-supplemented medium. In an attempt to establish the functional role of clusterin in the apoptotic cell death of ovarian follicles, the biosynthesis of clusterin in granulosa cells of healthy follicles was blocked by treatment of cells with antisense oligonucleotide to its cDNA. Treatment of granulosa cells with the antisense oligonucleotide resulted in an increase in the apoptotic cell death compared to the control. These findings indicate that depletion of clusterin can lead to the programmed cell death in ovary, suggesting a functional role for this protein in follicular atresia [121].

Luteolysis was induced in pregnant sows (45 days) by Cloprostenol (PGF2 alpha analogue) treatment. Clusterin expression increased in the corpora lutea of pregnant sows ovariectomized 0, 6, 12, 24, 48 or 72 (n = 3) h after the luteolytic stimulus; maximum values were observed 24-48 h after the treatment ($P < 0.01$). An opposite trend between clusterin mRNA expression and markers of luteal function, such as progesterone levels in the corpora lutea and plasma, and LHr mRNA expression levels, was observed; moreover, clusterin expression was positively correlated with the degree of genomic DNA fragmentation, a marker of occurring apoptosis ($P < 0.01$). This pattern may be important in regulating luteolysis by a switch between luteotrophic and apoptotic stimulus. Our data indicate that P4 levels decrease prior to the increase in clusterin mRNA and the drop in LHr mRNA expression; we may therefore hypothesize a split between functional and structural luteolysis as reported in other species [160].

BLOOD CELLS

Peripheral blood cells were assayed for clusterin expression. The protein was predominantly detectable in human platelets by immune cytochemistry. The content of clusterin was determined and amounts to 2.5 +/- 1.3 micrograms/10(9) platelets, thus representing about 2% of the blood pool. Clusterin purified from human platelets had the same molecular weight as plasma clusterin under nonreducing conditions and was composed of two disulfide-linked nonidentical subunits of the same size. Both preparations were sensitive to reduction yielding the two subunits of 35 Kd. In contrast to plasma clusterin, the platelet form was not complexed to apolipoprotein A-I. By immunogold labeling, alpha-granule localization of clusterin was observed. Complete release of platelet clusterin occurred at optimal doses of A23187, phorbol myristate acetate (PMA), and thrombin. Because clusterin mRNA was detected by hybridization in situ in bone marrow-derived megakaryocytes, platelet clusterin is most likely produced and packaged into alpha-granules during megakaryocyte development [36].

Atheromatous plaque development involves platelet aggregation and activation, and abundant clusterin protein was found in advanced human atheromatous lesions. Thus, platelet sequestration and activation may lead to the rapid deployment of clusterin into sites of vascular injury. It is hypothesized that platelet-derived clusterin participates in both short-term wound repair processes and chronic pathogenic processes at vascular interfaces [37].

ATHEROSCLEROSIS

Clusterin, a glycoprotein associated with subclasses of plasma high density lipoproteins (HDL), was found to accumulate in aortic lesions in a human subject with transplantation-associated arteriosclerosis and in mice fed a high-fat atherogenic diet. Foam cells present in mouse aortic valve lesions expressed clusterin mRNA, suggesting local synthesis contributes to clusterin's localization in atherosclerotic plaque [42].

Although clusterin was never observed in the normal aorta (ie, without any intimal lesions or intimal thickening), it was distributed not only in the intima but also in the media of aortas with diffuse, intimal thickening or atherosclerotic lesions. Double immunostaining with antibodies for clusterin and alpha-smooth muscle actin revealed clusterin deposition in smooth muscle cells (SMCs) or the aortic stroma in the vicinity of SMCs. The extent of clusterin distribution in the aortic wall increased with the degree of atherosclerosis development. In addition, the distribution pattern of clusterin was very similar to that of apoA-I and E. In situ hybridization with human clusterin cDNA demonstrated intense signals in cells scattered within the subendothelial space and medial SMCs of the aorta with advanced atherosclerosis but not in those of the normal aorta without intimal thickening. Furthermore, reverse transcriptase-polymerase chain reaction of the cultured human aortic SMCs revealed clusterin mRNA expression in these cells. The results indicate that clusterin in the aortic wall originates from not only clusterin circulated in the plasma but also clusterin produced by SMCs in the aortic wall. Considering the similarities of the distribution between clusterin and apo-A-I or E, it is hypothesized that clusterin possibly has a protective role against human atherosclerosis by its involvement with cholesterol transport from the aortic wall to the liver [96].

Mouse peritoneal macrophages were incubated with acetylated low-density lipoprotein (AcLDL) to produce foam cells containing cholesterol and cholesteryl esters. Incubation of the foam cells with physiological concentrations of purified clusterin led to a dose-dependent export of cholesterol. The appearance of cholesterol in the medium was associated predominantly with a decline in intracellular cholesteryl esters rather than intracellular free cho-

lesterol. The kinetics of cholesterol release to clusterin were similar to apo A-I, an established promoter of cholesterol efflux. Clusterin was also shown to induce phospholipid efflux from cells, whereas the cholesterol exported to the medium was associated with clusterin. Studies using foam cells from apo E-null mice showed that the cholesterol exported to the medium was independent of apo E production by the cells. These results present the first evidence that clusterin can promote cholesterol efflux from foam cells and indicates that it might have a function in cellular cholesterol homoeostasis in both normal and pathological situations [97].

Paraoxonase (Pon), clusterin, and apolipoprotein (apo) A-I accumulate in the artery wall during the development of atherosclerosis. In normal aortas (n = 6) there were low levels of extracellular Pon, clusterin, and apoA-I, immunoreactivity. The cytoplasm of smooth muscle cells in the media showed granular positivity for both Pon and apoA-I, indicating that these proteins were undergoing lysosomal degradation. This activity was also indicated by the presence of both intact and degradation products of Pon in smooth muscle cells as shown by Western blotting. With the progression of disease from fatty streaks (n = 3) to advanced atherosclerosis (n = 8) there was an increase in Pon, apoA-I, and clusterin immunoreactivity, indicating the increasing presence of these proteins with disease progression. These proteins are the components of a specific HDL subspecies that has been implicated in the prevention of peroxidative damage to phospholipids in LDL and membranes. The increase in Pon, clusterin, and apoA-I during the development of atherosclerosis may therefore represent a protective response to the oxidative stress associated with the development of atherosclerosis [80].

The effects of mildly oxidized LDL and atherosclerosis on the levels of two proteins associated with HDL; clusterin, and paraoxonase (PON) were studied. On an atherogenic diet, PON activity decreased by 52%, and apoJ levels increased 2.8-fold in fatty streak susceptible mice, C57BL/6J (BL/6), but not in fatty streak resistant mice, C3H/HeJ (C3H). Plasma PON activity was also significantly decreased, and clusterin levels were markedly increased in apolipoprotein E knockout mice on the chow diet, resulting in a 9.2-fold increase in the clusterin/PON ratio as compared to controls. Furthermore, a dramatic increase in the clusterin/PON ratio (over 100-fold) was observed in LDL receptor knockout mice when they were fed a 0.15%-cholesterol-enriched diet. Injection of mildly oxidized LDL (but not native LDL) into BL/6 mice (but not in C3H mice) on a chow diet resulted in a 59% decrease in PON activity ($P < 0.01$) and a 3.6-fold increase in clusterin levels ($P < 0.01$). When an acute phase reaction was induced in rabbits, or the rabbits were placed on an atherogenic diet, hepatic mRNA for clusterin was increased by 2.7-fold and 2.8-fold, respectively. Treatment of HepG2 cells in culture with mildly oxidized LDL (but not native LDL) resulted in reduced mRNA levels for PON (3.0-fold decrease) and increased mRNA levels for clusterin (2.0-fold increase). In normolipidemic patients with angiographically documented coronary artery disease who did not have diabetes and were not on lipid-lowering medication (n = 14), the total cholesterol/HDL cholesterol ratio was 3.1+/-0.9 as compared to 2.9+/-0.4 in the controls (n = 19). This difference was not statistically significant. In contrast, the clusterin/PON ratio was 3.0+/-0.4 in the patients compared to 0.72+/-0.2 in the controls ($P < 0.009$). In a subset of these normolipidemic patients (n = 5), the PON activity was low (48+/-6.6 versus 98+/-17 U/ml for controls; $P < 0.009$), despite similar normal HDL levels, and the HDL from these patients failed to protect against LDL oxidation in co-cultures of human artery wall cells. It is concluded that: (a) mildly oxidized LDL can induce an increased clusterin/PON ratio, and (b) the clusterin/PON ratio may prove to be a better predictor of atherosclerosis than the total cholesterol/HDL cholesterol ratio [77].

In a study analyzing the effects of the lecitin-chesterol acyltranferase gene knock-out, clusterin levels, rather than being decreased, were significantly ($P = 0.01$) higher (36%) in Lcat (-/-) than in Lcat (+/+) mice, and the clusterin/PON ratio was 3-fold greater in Lcat (-/-) than in Lcat (+/+) animals112.
A peptide was synthesized from D-amino acids corresponding to residues 113 to 122 in clusterin. D- [113-122]clusterin significantly improved the ability of plasma to promote cholesterol efflux and improved high-density lipoprotein (HDL) inflammatory properties for up to 48 hours after a single oral dose in apoE-null mice, whereas scrambled D- [113-122]clusterin did not. Oral administration of 125 microg/mouse/d of D- [113-122]clusterin reduced atherosclerosis in apoE-null mice (70.2% reduction in aortic root sinus lesion area, P=4.3 x 10(-13); 70.5% reduction by en face analysis, P=1.5 x 10(-6)). In monkeys, oral D- [113-122]clusterin rapidly reduced lipoprotein lipid hydroperoxides (LOOH) and improved HDL inflammatory properties. Adding 250 ng/mL of D-[113-122]clusterin (but not scrambled D- [113-122]clusterin) to plasma in vitro reduced LOOH and increased paraoxonase activity. Oral D- [113-122]clusterin significantly improves HDL inflammatory properties in mice and monkeys and inhibits lesion formation in apoE-null mice [165].

An insertion (I)/deletion (D) polymorphism was found within the clusterin gene, D/D subjects had significantly higher levels of total cholesterol and low-density lipoprotein (LDL)-cholesterol than I/I subjects in females but not in males. Female subjects with the D allele (D/D+I/D) had greater intima-media thickness of the carotid artery than I/I subjects. In a multiple logistic regression analysis, the D allele of 6316delT was detected as an independent predictor for the plaque prevalence. In conclusion, the clusterin gene polymorphism may contribute to the serum lipid levels and the progression of carotid atherosclerosis in hypertensive Japanese females [163].

PANCREAS

Exocrine Pancreas

Clusterin mRNA levels were strongly increased 6 h after pancreatitis induction. Maximal expression happened between 24-48 h and decreased progressively to undetectable levels at day 5. Clusterin mRNA was expressed with similar intensity in oedematous caerulein-induced pancreatitis and in response to various degrees of necrohaemorrhagic taurocholate-induced pancreatitis, indicating a maximal gene activity in all types of pancreatitis; in situ hybridization showed that the acinar cells and some ducts expressed clusterin mRNA. A single band of about 35-38 kDa was detected by western blot in pancreatic homogenates and in pancreatic juice from rats with acute pancreatitis, but not from control rats [101].

Clusterin is overexpressed in the pancreas at the onset of chronic pancreatitis in vivo and in cultured acinar cells in response to various stimuli in vitro, suggesting that clusterin is a defense mechanism of the exocrine pancreas [130].

Clusterin expression was examined throughout the process of pancreatic neogenesis in pancreatectomized rats. For in vitro analysis, duct cells were isolated from the rat pancreas and clusterin cDNA was transfected for its overexpression. Clusterin and its mRNA increased significantly in the early phase of regeneration, particularly at 1-3 days after pancreatectomy. Clusterin was transiently expressed in the differentiating acinar cells but faded afterwards. Interestingly, these clusterin cells were negative for PCNA (proliferating cell nuclear antigen), whereas most epithelial cells in ductules in the regenerating tissue showed extensive proliferative activity. Clusterin expression was also detected in some endocrine cells of the regenerating tissue. Transfection of clusterin cDNA into primary cultured duct cells resulted in a 2.5-fold increase in cell proliferation and induced transformation of non-differentiated duct cells into differentiated cells displaying cytokeratin immunoreactivity. Taken together, these results suggest that clusterin may play essential roles in the neogenic regeneration of pancreatic tissue by stimulating proliferation and differentiation of duct cells [157].

AR4-2J cells with modified expression levels of clusterin and in vivo studies in clusterin-deficient mice. AR4-2J cells were exposed to agents mimicking cell-stress during pancreatitis (cerulein, hydrogen peroxide, staurosporine or lysophosphatidylcholine). Clusterin-overexpressing AR4-2J cells showed higher viability after cell stress and accordingly reduced rates of apoptosis and lessened caspase-3 activation. Blockage of endogenous clusterin expression reduced viability and enhanced apoptosis. Presence of clusterin reduced NF-kappaB activation and expression of the NF-kappaB target genes TNF-alpha and MOB-1 under cell stress. Clusterin-deficient mice showed a more severe course of acute experimental pancreatitis with enhanced rates of apoptosis and inflammatory cell infiltration. We concluded that clusterin was protective during inflammation of exocrine pancreas because of its anti-apoptotic and anti-inflammatory functions [179].

Morphological studies have shown that copper deficiency-induced pancreatic involution in rats is secondary to apoptosis (M. S. Rao, A. V. Yeldandi, V. Subbarao, and J. K. Reddy, 1993, Am. J. Pathol. 142, 1952-1957). Northern blot analysis of total RNA revealed a marked increase in the expression of SGP-2 mRNA at 5 weeks followed by a gradual decrease at 6 and 7 weeks. These results further support that the mechanism of copper deficiency-induced pancreatic involution is through apoptosis [45].

Endocrine Pancreas

The expression of clusterin in pancreatic tissue of streptozotocin-treated rats which were undergoing extensive islet tissue reorganization due to degeneration of insulin beta cells was investigated. Clusterin was found in endocrine cells identified as glucagon-secreting alpha cells at the periphery of the islet. Using immunoelectron microscopy, clusterin-positive cells showed the typical ultrastructural features of pancreatic alpha cells. In addition, colocalization of clusterin and glucagon in the same secretory granules was shown by double immunogold labeling. These results imply that clusterin is a secretory molecule having endocrine and/or paracrine actions in parallel with glucagon.

Further, we noted that clusterin expression was increased in pancreatic alpha cells during the process of beta cell death upon streptozotocin injection. The increase was significant as early as 1-3 h after streptozotocin treatment prior to any morphological alteration of islet beta cell and any manifestation of hyperglycemia. The expression of clusterin was steady-stately up-regulated during the process of islet reorganization caused by streptozotocin-induced cytotoxic injury. Therefore, it is suggested that clusterin might be considered as a molecule induced by both embryonic development and drug-induced reorganization of the endocrine pancreas. Since clusterin expression is up-regulated in alpha cells, but not in beta cells undergoing degeneration, it may play a protective role against the cytotoxic insult [113].

A diabetogenic dose of streptozotocin injected in rats provoked an immediate degeneration of beta cells. In this model, islets showed increased clusterin expression with extensive proliferation of alpha cells but showed poor beta-cell replication. A subdiabetogenic dose of streptozotocin, however, led to the proliferation of beta cells with clusterin up-regulation. In streptozotocin-treated neonatal rats, up-regulation of clusterin was noted during beta-cell proliferation. In all experimental models, clusterin was expressed in alpha cells in close correlation with islet cell proliferation, higher transcription of insulin mRNA and MAPKs activation. Cell replication was increased by 31 % in the MIN6 cells transfected by the clusterin cDNA. Up-regulation of clusterin in alpha cells might induce beta-cell proliferation and thus restore their population after islet injury. We suggest that clusterin could be considered as a growth factor-like molecule stimulating islet-cell proliferation by paracrine action [139].

Isolated ductal tissue from rat pancreas was cultured it to develop epithelial cell explants for transfection of clusterin cDNA as well as for treatment of clusterin protein. The number of newly differentiated insulin cells increased 6.9-fold upon clusterin overexpression compared with controls. Ins1 mRNA and peptide levels were also increased. Furthermore, glucose-stimulated insulin secretion was observed in the differentiated insulin cells. These cells were immunoreactive for insulin and C-peptide, but negative for other islet hormones and for cytokeratin-20, which indicates a fully differentiated state. Insulin cell differentiation was also increased in a dose-dependent manner by treating duct cells in culture with clusterin, indicating a growth-factor-like action of clusterin in insulin cell differentiation. These results suggest that clusterin can be considered as a potential morphogenic factor that promotes differentiation of pancreatic beta cells [170].

Analysis of LC-FTICR proteomic data identified five candidate protein biomarkers of type 1 diabetes. alpha-2-Glycoprotein 1 (zinc), corticosteroid-binding globulin, and lumican were 2-fold up-regulated in type 1 diabetic samples relative to control samples, whereas clusterin and serotransferrin were 2-fold up-regulated in control samples relative to type 1 diabetic samples. Observed perturbations in the levels of all five proteins are consistent with the metabolic aberrations found in type 1 diabetes [191].

VASCULATURE

Smooth Muscle

Cultured porcine smooth muscle cells (SMC) undergo morphological and phenotypic modulation associated with a change from a substrate-attached monolayer culture to a nodular culture in which most of the cells are present in multicellular aggregations (nodules). During that transition from monolayer to nodular cell culture (> 8 days) the expression of clusterin mRNA and protein is increased. Clusterin expression continues in the nodular cell cultures and it is secreted at 0.3 micrograms/ml/24 h. These results demonstrate differential expression of SMC clusterin and suggest that clusterin has a functional role in SMC modulation [47].

Clusterin, at 10 microg/ml, clearly promotes vascular smooth Musile cells (VSMC) migration. In addition, a 15 amino acid synthetic peptide, representing amino acids 118-132 [KQTCMKFYARVCRSG] of the mature clusterin polypeptide, inhibits VSMC attachment to gelatinous substrate. Finally, clusterin appears to have a role in regulating endogenous clusterin expression in the clusterin negative clone. These results clearly establish that clusterin has functional role in VSMC nodule formation and support the conclusion that clusterin is a critical component of smooth muscle cell phenotypic modulation [128].

Clusterin mRNA was not detectable in noninjured aorta (control), began to be expressed at 6 hours after injury, showed a peak level at 24 hours (a 48-fold increase), gradually declined, and returned to the control level at 24 weeks. Western blot and immunohistochemistry demonstrated no expression of clusterin protein in noninjured aorta, an expression of clusterin at 2 days after balloon injury, and a peak level (a 55-fold increase) at 2 to 8 weeks. The expression of clusterin protein continued until 24 weeks after injury. In situ hybridization revealed that clusterin mRNA was expressed in smooth muscle

cells (SMCs) of media at 2 days after injury and in SMCs of media and neointima at 2 weeks. To analyze the function of clusterin, stably transfected rabbit SMCs were created. The expression of clusterin stimulated proliferation and migration of SMCs. Clusterin is dramatically induced in media and neointima after vascular injury, suggesting that clusterin contributes to restenosis after angioplasty [136].

Intimal Cells

Clusterin expression was evaluated by immunohistochemistry and Western blotting in human arteries and rat aortas. In human diffuse myointimal thickening, clusterin was detected in cell cytoplasm and extracellular space, whereas it was practically absent in the media. In rat aortas 15 days after ballooning, intimal cells (IT cells) overexpressed s-clusterin and n-clusterin, the latter mainly in the inner neointima; clusterin expression decreased at 60 days. In vitro, IT cells maintained high clusterin expression and its antisense markedly reduced proliferation and increased apoptosis. Western blotting showed that all-trans retinoic acid-induced proliferative arrest and increased alpha-smooth muscle actin expression did associate to s-clusterin and B-myb reduction, whereas bax-related apoptosis was associated to a shift from the s-clusterin to n-clusterin isoform. Clusterin overexpression characterized neointimal SMCs; s-clusterin expression decreased in IT cells during all-trans retinoic acid-induced proliferative arrest and redifferentiation, whereas n-clusterin overexpression was characteristic of apoptosis. Clusterin was detected in human arterial myointimal thickening and absent in the underlying media. Rat neointimal cells overexpressed clusterin and clusterin antisense oligonucleotide reduced proliferation and increased apoptosis. All-trans retinoic acid-induced proliferative arrest showed association with s-clusterin reduction and n-clusterin overexpression with apoptosis, supporting a different biological role of these isoforms [162].

Endothelial cells

Clusterin inhibits HUVEC migration and adhesion. By altering endothelial function during vascular injury, clusterin appears to regulate, in part, the early development of intimal hyperplasia after prosthetic arterial grafting [133].

When Human retinal endothelial cells (HRECs) were exposed to oxygen-glucose deprivation (OGD), clusterin expression increased, whereas von Willebrand factor (vWF), occludin, and zonula occludens (ZO-1) markedly decreased. Interestingly, loss of tight junction proteins and death of HRECs in OGD conditions were restored by clusterin treatment. These results suggest that the enhanced clusterin in OGD conditions may play a protective role against ischemia-induced tight junction protein loss and HRECs death [186].

GIT

As compared with controls, a strongly enhanced expression of clusterin was found in Crohn disease (CD) tissues, correlating with disease activity. Immunohistochemistry and in situ hybridization analysis revealed foci of crypts almost completely lined by clusterin expressing enterocytes in CD, a feature that was never seen in controls. Such crypts appeared especially within the morphologically intact mucosa apart from erosive or ulcerative lesions. Besides epithelia, clusterin was also expressed by inflammatory mononuclear cells. Enhanced expression of clusterin by crypt epithelia might reflect a cytoprotective function of the protein in order to prevent further injury of the intestinal mucosal barrier in CD [134].

Connective Tissue

The expression of clusterin mRNA was up-regulated in early osteoarthritic vs normal cartilage when analysed by microarray analysis. Using in situ hybridization, chondrocytes of normal cartilage expressed moderate levels of clusterin. Upper mid-zone chondrocytes in cartilage with early stages of osteoarthritic disease expressed high levels of clusterin mRNA. In advanced osteoarthritic cartilage, the overall expression of clusterin was reduced. The induction of clusterin has been associated with a variety of disease states where it appears to provide a cytoprotective effect. The increased expression of clusterin mRNA in the early stages of osteoarthritis (OA) may reflect an attempt by the chondrocytes to protect and repair the tissue. In contrast, the decrease in clusterin mRNA in the advanced osteoarthritic cartilage accompanies the final degenerative stages of the disease. An understanding of the expression of clusterin in osteoarthritis may allow consideration of this protein as a marker for cartilage changes in this chronic degenerative condition [141].

In synovial tissue, the protein was predominantly expressed by synoviocytes and it was detected in synovial fluids. Both full-length and spliced isoform CLU mRNA levels of expression were lower in reumatoid arthritis (RA) tissues compared with osteoarthritis (OA) and healthy synovium. In synovium and in cultured FLS, the overexpression of clusterin concerned all protein isoforms in OA whereas in RA, the intracellular forms of the protein were

barely detectable. Transgenic overexpression of clusterin in RA FLS promoted apoptosis within 24 h. It was observed that clusterin knockdown with small interfering RNA promoted IL-6 and IL-8 production. Clusterin interacted with phosphorylated IkappaBalpha. Differential expression of clusterin by OA and RA FLS appeared to be an intrinsic property of the cells. Expression of intracellular isoforms of CLU is differentially regulated between OA and RA. It was proposed that in RA joints, high levels of extracellular CLU and low expression of intracellular CLU may enhance NF-kappaB activation and survival of the synoviocytes [177].

Fibroblasts

Human diploid fibroblasts (HDFs) exposed to subcytotoxic stresses under H2O2, tert-butylhydroperoxide (t-BHP), and ethanol (EtOH) undergo stress-induced premature senescence (SIPS) characterized by many biomarkers of HDFs replicative senescence. Among these biomarkers are a growth arrest, an increase in the senescence-associated beta-galactosidase activity, a senescent morphology, an overexpression of p21waf-1 and the subsequent inability to phosphorylate pRb, the presence of the common 4977-bp mitochondrial deletion, and an increase in the steady-state level of several senescence-associated genes such as clusterin. Clusterin has been described as a survival gene against cytotoxic stress. In order to study whether clusterin would be protective against cytotoxicity SIPS and replicative senescence in human fibroblasts, a full-length complementary deoxyribonucleic acid of clusterin was transfected into WI-38 HDFs and SV40-transformed WI-38 HDFs. The overexpression of clusterin resulted in an increased cell survival after t-BHP and EtOH stresses at cytotoxic concentrations. In addition, when WI-38 HDFs were exposed to 5 subcytotoxic stresses with EtOH or t-BHP, in conditions that were previously shown to induce SIPS, a lower induction of 2 biomarkers of SIPS was observed in HDFs overexpressing clusterin. No effect of clusterin overexpression was observed on the proliferative life span of HDFs, even if clusterin overexpression triggered osteonectin (SPARC) overexpression, which was shown to decrease the mitogenic potential of platelet-derived growth factor but not of other common growth-inducing conditions. Clusterin senescence-related overexpression is proposed to have antiapoptotic rather than antiproliferative effects [144].

Fibroblasts were coincubated with conditioned medium and cigarette smoke extract, and bronchial biopsy specimens obtained from nonsmokers, smokers, and ex-smokers were analyzed by immunohistochemistry. At concentrations of 2.5 and 5.0%, cigarette smoke extract induced oxidative stress. It also markedly increased the expression of two clusterin isoforms (60 and 76-80 kD) and the 76-80-kD isoform was secreted in the incubation medium. Coincubation of fibroblasts with conditioned medium significantly decreased the cellular oxidation caused by the cigarette smoke extract. Immunohistochemical analysis of clusterin on bronchial biopsy specimens obtained from smokers and ex-smokers showed localization of clusterin mainly in the submucosa. I was concluded that clusterin may have a protective effect against cigarette smoke-induced oxidative stress in lung fibroblasts [173].

SKIN

Elastotic material of sun-damaged skin (solar elastosis) revealed a strong staining for clusterin. Because of the striking co-localization of clusterin with abnormal elastic material, the interaction of clusterin with elastin in vitro was investigated. A chaperone assay was established in which elastin was denatured by UV irradiation in the absence or presence of clusterin. This assay demonstrated that clusterin exerted a chaperone-like activity and effectively inhibited UV-induced aggregation of elastin. The interaction of both proteins was further analyzed by electron microscopy, size exclusion chromatography, and mass spectrometry, in which clusterin was found in a stable complex with elastin after UV exposure [187].

PREECLAMPSIA NAD HYPERTENSION

866C-->T polymorphism in exon 5 and a rare variant 1061C-->T in exon 7 was identified and it was found that the CT genotype and T-allele frequencies in preeclampsia (PE) (CT genotype 26%; T allele 13%) and essential hypertension (EH) (CT genotype 28.57%; T allele 17.14%) were significantly lower than that in the control group (CT geno type 50%; T allele 27%). The results suggested that the 866C-->T polymorphism might be associated with PE and EH. It is plausible that apo J may play a certain role in the predisposition to PE and EH [164].

CANCER

It was shown taht in patients with renal clear cell carcinoma gp80 mRNA is 3-fold overexpressed in tissue of the tumors compared with adjacent non-tumor tissue [39].

CLUSTERIN AND LEPTIN

Complex formation between clusterin and leptin by several approaches was demonstrated and shown that the binary complex retains the ability to transduce the leptin signal via binding to the leptin receptor and activation of the Janus kinase/signal transducer and activator of transcription pathway. The interaction of leptin with clusterin does not require additional serum components. Furthermore, and importantly for modulation of the bioactivity of leptin, uptake of leptin present in the complex can be mediated by members of the low density lipoprotein (LDL) receptor family, i.e., apolipoprotein receptor type-2 and the very LDL receptor, which here are shown to efficiently endocytose both free and leptin-associated clusterin. Thus, bioavailability of leptin at a given tissue site may be determined by the levels of clusterin and/or by the relative distribution of certain relatives of the LDL receptor vis-à-vis active leptin receptors [152].

CLUSTERIN AND GHRELIN

an immobilized form of ghrelin specifically binds a species of high density lipoprotein associated with the plasma esterase, paraoxonase, and clusterin. Both free ghrelin and paraoxon, a substrate for paraoxonase, can inhibit this interaction. An endogenous species of ghrelin is found to co-purify with high density lipoprotein during density gradient centrifugation and subsequent gel filtration. This interaction links the orexigenic peptide hormone ghrelin to lipid transport and metabolism. Furthermore, the interaction of the esterified hormone ghrelin with a species of HDL containing an esterase suggests a possible mechanism for the conversion of ghrelin to des-acyl ghrelin [154].

CLUSTERIN AND MATRIX METALLOPROTEINASE 26

MT6-MMP/MMP-25 is the latest member of the membrane-type matrix metalloproteinase (MT-MMP) subgroup in the MMP family and is expressed in neutrophils and some brain tumors. The proteolytic activity of MT6-MMP has been studied using recombinant catalytic fragments and shown to degrade several components of the extracellular matrix. However, the activity is possibly modulated further by the C-terminal hemopexin-like domain, because some MMPs are known to interact with other proteins through this domain. To explore the possible function of this domain, a recombinant MT6-MMP with the hemopexin-like domain was purified as a soluble form using a Madin-Darby canine kidney cell line as a producer. Mature and soluble MT6-MMP processed at the furin motif was as a 45-kDa protein together with a 46-kDa protein having a single cleavage in the hemopexin-like domain. Interestingly, 73- and 70-kDa proteins were co-purified with the soluble MT6-MMP by forming stable complexes. They were identified as clusterin, a major component of serum, by N-terminal amino acid sequencing. MT1-MMP that also has a hemopexin-like domain did not form a complex with clusterin. MT6-MMP forming a complex with clusterin was detected in human neutrophils as well. The enzyme activity of the soluble MT6-MMP was inactive in the clusterin complex. Purified clusterin was inhibitory against the activity of soluble MT6-MMP. On the other hand, it had no effect on the activities of MMP-2 and soluble MT1-MMP. Because clusterin is an abundant protein in the body fluid in tissues, it may act as a negative regulator of MT6-MMP in vivo [153].

CLUSTERIN IN CIRCULATION

Atherosclerosis and Diabetes

By combining a sandwich ELISA assay and immunoblotting analysis a measurable increase of clusterin serum levels with age in males was demonstrated and provide evidence that, as compared to healthy donors, the serum clusterin amount increases significantly in diabetic type II patients and in patients suffering from either a developing coronary heart disease, or myocardial infarction. The highest serum clusterin levels were found during myocardial infarction but no correlation was observed with the number of vessels with documented atherosclerotic damage. Clusterin accumulation in serum is probably coupled to a generalized stress mediated induction mechanism that is

specifically related to certain diseases; moreover these data raise the possibility that elevated clusterin levels in serum may represent a strong indication of vascular damage [148].

Serum clusterin was 52.8+/-0.8 microg/mL (mean+/-SEM; range, 36.0-84.3 microg/mL; n=92) in healthy Japanese men, and 49.3+/-0.5 microg/mL (34.5-72.8; n=241) in healthy Japanese women. Multiple regression of these data and results from 67 men with coronary heart disease (CHD) showed that clusterin concentration was unrelated to age, sex or body mass index, but was positively related to serum PON1 ($p<0.001$) and apo B ($p<0.02$) concentrations. In women, it was also positively related to blood glucose ($p<0.02$). After adjusting for its associations with covariates, serum clusterin averaged 5.4 microg/mL lower in CHD men than in controls ($p<0.003$). Type 2 diabetics had higher clusterin concentrations (men, 83.1+/-3.4 microg/mL, n=64; women, 64.0+/-2.3 microg/mL, n=46) than healthy men and women ($p<0.001$). In these Type 2 diabetics, clusterin concentration was unrelated to PON1 concentration, but was positively related to blood glucose ($p<0.01$). After adjustment for its relation to blood glucose, the mean clusterin concentration was similar in diabetics and healthy subjects. These findings suggest that clusterin may be anti-atherogenic in humans, and that its concentration is raised by Type 2 diabetes [178].

Liver Cirrhosis

Clusterin and vitronectin were quantified in serum from patients suffering from alcoholic liver cirrhosis (n = 83), and in serum-free culture supernatants from the hepatoma cell line HepG2. The median clusterin concentration was 0.20 g/l in cirrhosis and 0.37 g/l in the controls, whereas corresponding vitronectin values were 0.19 and 0.26 g/l, respectively. The concentration of both proteins showed significant correlation ($p < 0.0001$) with disease severity and with established plasma markers of hepatic synthetic function, such as albumin and prothrombin complex. The clusterin level, but not the vitronectin level, correlated with survival ($p = 0.005$). The rates of synthesis of clusterin, vitronectin and C3 from HepG2 cells were 0.02, 0.21 and 1.9 micrograms/10(6) cells/24 h, respectively. It is concluded that clusterin (as vitronectin and C3) is mainly produced in the liver and may be a useful marker in the evaluation of severity of liver disease and prognosis of patients with alcoholic cirrhosis [63].

Alcohol Consumption

(1)The sialylation index of clusterin (moles sialic acid per mole clusterin protein) in rats administered ethanol for 4, 6, and 8 weeks and a gradual withdrawal and a follow-up abstinence for 1, 2, and 4 weeks; and (2) enzymatic activities of hepatic sialyltransferase and plasma sialidase during the same periods of alcohol treatment and abstinence in rats were determined. Although no significant differences in the clusterin sialylation index between rats of the control and ethanol groups were found at the 4th week of alcohol treatment, a highly significant loss of 24% ($p < 0.001$) and 44% ($p < 0.001$) was found after 6 and 8 weeks, respectively, of alcohol feeding of these animals. Furthermore, a significant recovery of 38% ($p < 0.001$), 78% ($p < 0.001$), 84% ($p < 0.001$) and 96% ($p < 0.001$) in the sialylation index of clusterin were found, respectively, during withdrawal and 1, 2, and 4 weeks of subsequent alcohol abstinence in these animals. These changes in the sialic acid content of clusterin were accompanied by a similar pattern of changes in the enzyme activities of hepatic sialyltransferase and plasma sialidase in animals undergoing chronic ethanol treatment, withdrawal, and abstinence periods. The analysis of the sialylation index of clusterin seems to be a simple and feasible method to use to evaluate the extent of ethanol exposure110.
Chronic alcohol consumption impairs the hepatic sialylation of a number of glycoproteins by specifically down-regulating Gal-beta-1,4GlcNAc alpha2,6-sialyltransferase mRNA. It was found that chronic ethanol consumption markedly inhibits hepatic sialylation of clusterin. Because the sialic-acid index of Apo J (SIJ; moles of sialic acid per mole of Apo J protein) is approximately seven times more than that for transferrin (28 vs. 4), it was evaluated whether plasma SIJ would be an even more sensitive marker for chronic ethanol consumption than CDT in both rats and human subjects. The method involved immunoaffinity purification of plasma HDL-Apo J, followed by its sialic acid determination. It was found that chronic ethanol feeding resulted in loss of sialic acid residues of plasma HDL-Apo J in rats. This loss of sialic acid was positively correlated with both amount and duration of ethanol treatment. In human subjects, an intake of about 60 g of alcohol for 30 days led to almost 50% ($P <.01$) depletion of sialic acid from plasma HDL-Apo J. Further, it was established that there was a positive correlation of alteration in SIJ with alcohol consumption, detoxification, abstinence, and relapse in human alcohol-dependent patients (sensitivity, 90%-92%). In addition, plasma SIJ was decreased by 50%-57% ($P <.01$) in both male and female alcohol-dependent subjects. It was suggest that plasma SIJ can be used as a viable marker for early detection of chronic alcohol consumption in human beings [140].

Systemic Lupus Erythematosus

The levels of serum clusterin were measured by ELISA in 80 patients with SLE (76 female, 4 male). Clinical and serological information was gathered on 115 visits. Overall disease activity scores were determined using the Systemic Lupus Activity Measure-Revised. Serum clusterin levels were significantly decreased in patients with SLE and correlated inversely with disease activity ($p < 0.00001$). Low clusterin levels were significantly associated with skin ulcers ($p < 0.0001$), loss of hair ($p = 0.002$), proteinuria ($p = 0.018$), low platelet count ($p = 0.03$), and arthritis ($p < 0.0001$). The clusterin levels did not correlate with either systemic complement consumption, as measured by C3 or C4, or with prednisone use. A highly significant correlation was observed between low levels of serum clusterin and a number of SLE disease features. This deficiency of clusterin could directly or indirectly affect the disease process. Individuals lacking sufficient amounts of clusterin systemically likely have poor control of antibody mediated inflammation at sites of apoptosis where autoantigens are exposed [107].

Peeclampsia

Immunoassays showed that clusterin levels in the 80 preeclamptic women were significantly higher than those in the 80 controls (mean +/- SD; 1.62 +/- 0.46 times reference level in preeclamptic women vs. 1.30 +/- 0.46 times reference level in controls, $P < 0.001$) [159].

Prostate Cancer

The average clusterin level in serum was reported to be 101+/-42 microg/ml (n=96). There was no correlation to age or serum cholesterol levels. Analysis of serum clusterin levels in patients with newly diagnosed prostate cancer (n=5), hormone responsive tumors (n=5), and hormone refractory disease (n=5), demonstrated that no significant changes in serum clusterin levels accompany the progression of prostatic disease, or response to hormone therapy [132].

CLUSTERIN IN URINE

Urinary excretion of clusterin after bilateral renal ischemia, in the (cy/ +) rat model of autosomal-dominant polycystic kidney disease and in the FHH rat model of focal segmental glomerulosclerosis was measured. After bilateral renal ischemia, the urinary excretion of clusterin paralleled the excretion of total protein and albumin and correlated with the extent of tubular damage. Male (cy/ +) rats, but not female (cy/ +) rats, excreted more clusterin than age-matched (+/ +) rats, a finding consistent with the more rapid course of the disease in males. FHH rats presented with pronounced proteinuria and albuminuria but did not excrete increased levels of clusterin. Urinary clusterin levels could therefore serve as a valuable marker for the severity of tubular damage. Furthermore, clusterin may also help to differentiate between tubular and glomerular forms of proteinuria [147].

Urine values of clusterin were higher in individuals with bladder cancer (197.2 vs 67.7, p=0.0007). Sensitivity for bladder cancer detection was 49% and specificity 92% (AUC 0.75, LR+ 6.1, PPV+ 84%); diagnostic efficacy was sufficient. Urine clusterin could be a laboratory marker of bladder cancer [175].

CLUSTERIN IN CEREBROSPINAL FLUID

Clusterin is significantly increased in cerebrospinal fluid from Alzheimer patients compared to unaffected controls, supporting that clusterin might be involved in the pathogenesis of Alzheimer's disease. However, the individual clusterin levels overlap between the two groups, and thus cerebrospinal fluid clusterin measurement is not suitable as a biochemical marker in the diagnosis of Alzheimer's disease [171].

A total of 164 cerebrospinal fluid (CSF) samples taken from neurological patients were classed into four groups according to the clinical diagnosis: multiple sclerosis (MScl, n = 44), clinically isolated syndrome of demyelination (CIS, n = 40), other inflammatory neurological disease (OIND, n = 26) and other neurological disease (OND, n = 54). Three differentially expressed proteins in the comparison of MScl vs. OND were identified: chromogranin A, a potential marker for neurodegeneration; and two important factors in complement-mediated inflammatory reaction, clusterin and complement C3 [193].

RELATED PRODUCTS

REFERENCES

1. Collard MW, Griswold MD.
Biosynthesis and molecular cloning of sulfated glycoprotein 2 secreted by rat Sertoli cells.
Biochemistry. 1987 Jun 16;26(12):3297-303

2. Cheng CY, Mathur PP, Grima J.
Structural analysis of clusterin and its subunits in ram rete testis fluid.
Biochemistry. 1988 May 31;27(11):4079-88

3. de Silva HV, Stuart WD, Duvic CR, Wetterau JR, Ray MJ, Ferguson DG, Albers HW, Smith WR, Harmony JA.
A 70-kDa apolipoprotein designated ApoJ is a marker for subclasses of human plasma high density lipoproteins.
J Biol Chem. 1990 Aug 5;265(22):13240-7.

4. de Silva HV, Stuart WD, Park YB, Mao SJ, Gil CM, Wetterau JR, Busch SJ, Harmony JA.
Purification and characterization of apolipoprotein J.
J Biol Chem. 1990 Aug 25;265(24):14292-7

5. Bettuzzi S, Troiano L, Davalli P, Tropea F, Ingletti MC, Grassilli E, Monti D, Corti A, Franceschi C.
In vivo accumulation of sulfated glycoprotein 2 mRNA in rat thymocytes upon dexamethasone-induced cell death.
Biochem Biophys Res Commun. 1991 Mar 29;175(3):810-5

6. Burkey BF, deSilva HV, Harmony JA.
Intracellular processing of apolipoprotein J precursor to the mature heterodimer.
J Lipid Res. 1991 Jun;32(6):1039-48

7. Connor J, Buttyan R, Olsson CA, D'Agati V, O'Toole K, Sawczuk IS.
SGP-2 expression as a genetic marker of progressive cellular pathology in experimental hydronephrosis.
Kidney Int. 1991 Jun;39(6):1098-103.

8. Eddy AA, Fritz IB.
Localization of clusterin in the epimembranous deposits of passive Heymann nephritis.
Kidney Int. 1991 Feb;39(2):247-52.

9. Rosenberg ME, Paller MS.
Differential gene expression in the recovery from ischemic renal injury.
Kidney Int. 1991 Jun;39(6):1156-61.

10. Jenne DE, Lowin B, Peitsch MC, Böttcher A, Schmitz G, Tschopp J.
Clusterin (complement lysis inhibitor) forms a high density lipoprotein complex with apolipoprotein A-I in human plasma.
J Biol Chem. 1991 Jun 15;266(17):11030-6

11. Sylvester SR, Morales C, Oko R, Griswold MD.
Localization of sulfated glycoprotein-2 (clusterin) on spermatozoa and in the reproductive tract of the male rat.
Biol Reprod. 1991 Jul;45(1):195-207

12. Harding MA, Chadwick LJ, Gattone VH 2nd, Calvet JP.
The SGP-2 gene is developmentally regulated in the mouse kidney and abnormally expressed in collecting duct cysts in polycystic kidney disease.
Dev Biol. 1991 Aug;146(2):483-90.

13. Leibovitch I, Buttyan R.
Sulfated glycoprotein-2 induced endogenous resistance to ischemia and reperfusion injury in the seminiferous tubules.
Am J Reprod Immunol. 1991 Oct;26(3):114-7

14. Grassilli E, Bettuzzi S, Monti D, Ingletti MC, Franceschi C, Corti A.
Studies on the relationship between cell proliferation and cell death: opposite patterns of SGP-2 and ornithine decarboxylase mRNA accumulation in PHA-stimulated human lymphocytes.
Biochem Biophys Res Commun. 1991 Oct 15;180(1):59-63.

15. Aulitzky WK, Schlegel PN, Wu DF, Cheng CY, Chen CL, Li PS, Goldstein M, Reidenberg M, Bardin CW.
Measurement of urinary clusterin as an index of nephrotoxicity.
Proc Soc Exp Biol Med. 1992 Jan;199(1):93-6.

16. Kirszbaum L, Bozas SE, Walker ID.
SP-40,40, a protein involved in the control of the complement pathway, possesses a unique array of disulphide bridges.
FEBS Lett. 1992 Feb 3;297(1-2):70-6.

17. Ledda-Columbano GM, Coni P, Faa G, Manenti G, Columbano A.
Rapid induction of apoptosis in rat liver by cycloheximide.
Am J Pathol. 1992 Mar;140(3):545-9.

18. Correa-Rotter R, Hostetter TH, Manivel JC, Eddy AA, Rosenberg ME.
Intrarenal distribution of clusterin following reduction of renal mass.
Kidney Int. 1992 Apr;41(4):938-50.

19. Cyr DG, Robaire B.
Regulation of sulfated glycoprotein-2 (clusterin) messenger ribonucleic acid in the rat epididymis.
Endocrinology. 1992 Apr;130(4):2160-6.

20. Kaynard AH, Periman LM, Simard J, Melner MH.
Ovarian 3 beta-hydroxysteroid dehydrogenase and sulfated glycoprotein-2 gene expression are differentially regulated by the induction of ovulation, pseudopregnancy, and luteolysis in the immature rat

21. Schumer M, Colombel MC, Sawczuk IS, Gobé G, Connor J, O'Toole KM, Olsson CA, Wise GJ, Buttyan R.
Morphologic, biochemical, and molecular evidence of apoptosis during the reperfusion phase after brief periods of renal ischemia.
Am J Pathol. 1992 Apr;140(4):831-8.

22. Pignataro OP, Feng ZM, Chen CL.
Cyclic adenosine 3',5'-monophosphate negatively regulates clusterin gene expression in Leydig tumor cell lines.
Endocrinology. 1992 May;130(5):2745-50.

23. Kangasniemi M, Cheng CY, Toppari J, Grima J, Stahler M, Bardin CW, Parvinen M.
Basal and FSH-stimulated steady state levels of SGP-2, alpha 2-macroglobulin, and testibumin in culture media of rat seminiferous tubules at defined stages of the epithelial cycle.

24. Mattmueller DR, Hinton BT.
Clusterin (SGP-2) in epididymal luminal fluid and its association with epididymal spermatozoa in androgen-deprived rats.
Mol Reprod Dev. 1992 May;32(1):73-80.

25. French LE, Tschopp J, Schifferli JA.
Clusterin in renal tissue: preferential localization with the terminal complement complex and immunoglobulin deposits in glomeruli.
Clin Exp Immunol. 1992 Jun;88(3):389-93.

26. Stuart WD, Krol B, Jenkins SH, Harmony JA.
Structure and stability of apolipoprotein J-containing high-density lipoproteins.
Biochemistry. 1992 Sep 15;31(36):8552-9.

27. Burkey BF, Stuart WD, Harmony JA.
Hepatic apolipoprotein J is secreted as a lipoprotein.
J Lipid Res. 1992 Oct;33(10):1517-26.

28. Choi-Miura NH, Takahashi Y, Nakano Y, Tobe T, Tomita M.
Identification of the disulfide bonds in human plasma protein SP-40,40 (apolipoprotein-J).
J Biochem. 1992 Oct;112(4):557-61.

29. Wilson MR, Easterbrook-Smith SB.
Clusterin binds by a multivalent mechanism to the Fc and Fab regions of IgG.
Biochim Biophys Acta. 1992 Oct 20;1159(3):319-26.

30. French LE, Sappino AP, Tschopp J, Schifferli JA.
Distinct sites of production and deposition of the putative cell death marker clusterin in the human thymus.
J Clin Invest. 1992 Nov;90(5):1919-25.

31. Pearse MJ, O'Bryan M, Fisicaro N, Rogers L, Murphy B, d'Apice AJ.
Differential expression of clusterin in inducible models of apoptosis.
Int Immunol. 1992 Nov;4(11):1225-31.

32. Schlegel PN, Matthews GJ, Cichon Z, Aulitzky WK, Cheng CY, Chen CL, Saso L, Goldstein M, Jänne OA, Bardin CW, et al.
Clusterin production in the obstructed rabbit kidney: correlations with loss of renal function.
J Am Soc Nephrol. 1992 Nov;3(5):1163-71.

33. Correa-Rotter R, Hostetter TH, Nath KA, Manivel JC, Rosenberg ME.
Interaction of complement and clusterin in renal injury.
J Am Soc Nephrol. 1992 Nov;3(5):1172-9.

34. Aronow BJ, Lund SD, Brown TL, Harmony JA, Witte DP.
Apolipoprotein J expression at fluid-tissue interfaces: potential role in barrier cytoprotection.
Proc Natl Acad Sci U S A. 1993 Jan 15;90(2):725-9.

35. Carone FA, Jin H, Nakamura S, Kanwar YS.
Decreased synthesis and delayed processing of sulfated glycoproteins by cells from human polycystic kidneys.
Lab Invest. 1993 Apr;68(4):413-8.

36. Tschopp J, Jenne DE, Hertig S, Preissner KT, Morgenstern H, Sapino AP, French L.
Human megakaryocytes express clusterin and package it without apolipoprotein A-1 into alpha-granules.
Blood. 1993 Jul 1;82(1):118-25.

37. Witte DP, Aronow BJ, Stauderman ML, Stuart WD, Clay MA, Gruppo RA, Jenkins SH, Harmony JA.
Platelet activation releases megakaryocyte-synthesized apolipoprotein J, a highly abundant protein in atheromatous lesions.
Am J Pathol. 1993 Sep;143(3):763-73

38. Väkevä A, Laurila P, Meri S.
Co-deposition of clusterin with the complement membrane attack complex in myocardial infarction.
Immunology. 1993 Oct;80(2):177-82.

39. Parczyk K, Pilarsky C, Rachel U, Koch-Brandt C.
Gp80 (clusterin; TRPM-2) mRNA level is enhanced in human renal clear cell carcinomas.
J Cancer Res Clin Oncol. 1994;120(3):186-8.

40. Kelso GJ, Stuart WD, Richter RJ, Furlong CE, Jordan-Starck TC, Harmony JA.
Apolipoprotein J is associated with paraoxonase in human plasma.
Biochemistry. 1994 Jan 25;33(3):832-9.

41. French LE, Wohlwend A, Sappino AP, Tschopp J, Schifferli JA.
Human clusterin gene expression is confined to surviving cells during in vitro programmed cell death.
J Clin Invest. 1994 Feb;93(2):877-84.

42. Jordan-Starck TC, Lund SD, Witte DP, Aronow BJ, Ley CA, Stuart WD, Swertfeger DK, Clayton LR, Sells SF, Paigen B, et al.
Mouse apolipoprotein J: characterization of a gene implicated in atherosclerosis.
J Lipid Res. 1994 Feb;35(2):194-210.

43. Saunders JR, Aminian A, McRae JL, O'Farrell KA, Adam WR, Murphy BF.
Clusterin depletion enhances immune glomerular injury in the isolated perfused kidney.
Kidney Int. 1994 Mar;45(3):817-27

44. Dvergsten J, Manivel JC, Correa-Rotter R, Rosenberg ME.
Expression of clusterin in human renal diseases.
Kidney Int. 1994 Mar;45(3):828-35.

45. Ide H, Yeldandi AV, Reddy JK, Rao MS.
Increased expression of sulfated glycoprotein-2 and DNA fragmentation in the pancreas of copper-deficient rats.
Toxicol Appl Pharmacol. 1994 May;126(1):174-7.

46. French LE, Sappino AP, Tschopp J, Schifferli JA.
Clusterin gene expression in the rat thymus is not modulated by dexamethasone treatment.
Immunology. 1994 Jun;82(2):328-31.

47. Thomas-Salgar S, Millis AJ.
Clusterin expression in differentiating smooth muscle cells.
J Biol Chem. 1994 Jul 8;269(27):17879-85.

48. O'Bryan MK, Murphy BF, Liu DY, Clarke GN, Baker HW.
The use of anticlusterin monoclonal antibodies for the combined assessment of human sperm morphology and acrosome integrity.
Hum Reprod. 1994 Aug;9(8):1490-6.

49. Rosemblit N, Chen CL.
Regulators for the rat clusterin gene: DNA methylation and cis-acting regulatory elements.
J Mol Endocrinol. 1994 Aug;13(1):69-76.

50. Nath KA, Dvergsten J, Correa-Rotter R, Hostetter TH, Manivel JC, Rosenberg ME.
Induction of clusterin in acute and chronic oxidative renal disease in the rat and its dissociation from cell injury.
Lab Invest. 1994 Aug;71(2):209-18.

51. Hardardóttir I, Kunitake ST, Moser AH, Doerrler WT, Rapp JH, Grünfeld C, Feingold KR.
Endotoxin and cytokines increase hepatic messenger RNA levels and serum concentrations of apolipoprotein J (clusterin) in Syrian hamsters.
J Clin Invest. 1994 Sep;94(3):1304-9.

52. Rosenberg ME, Manivel JC, Carone FA, Kanwar YS.
Genesis of renal cysts is associated with clusterin expression in experimental cystic disease.
J Am Soc Nephrol. 1995 Mar;5(9):1669-74.

53. Brown TL, Moulton BC, Baker VV, Mira J, Harmony JA.
Expression of apolipoprotein J in the uterus is associated with tissue remodeling.
Biol Reprod. 1995 May;52(5):1038-49.

54. Chung KH, Gomez RA, Chevalier RL.
Regulation of renal growth factors and clusterin by AT1 receptors during neonatal ureteral obstruction.
Am J Physiol. 1995 Jun;268(6 Pt 2):F1117-23.

55. Sensibar JA, Sutkowski DM, Raffo A, Buttyan R, Griswold MD, Sylvester SR, Kozlowski JM, Lee C.
Prevention of cell death induced by tumor necrosis factor alpha in LNCaP cells by overexpression of sulfated glycoprotein-2 (clusterin).
Cancer Res. 1995 Jun 1;55(11):2431-7.

56. Kounnas MZ, Loukinova EB, Stefansson S, Harmony JA, Brewer BH, Strickland DK, Argraves WS.
Identification of glycoprotein 330 as an endocytic receptor for apolipoprotein J/clusterin.
J Biol Chem. 1995 Jun 2;270(22):13070-5. Erratum in: J Biol Chem 1995 Sep 29;270(39):23234

57. Rosenberg ME, Silkensen J.
Clusterin: physiologic and pathophysiologic considerations.
Int J Biochem Cell Biol. 1995 Jul;27(7):633-45. Review.

58. Darby IA, Hewitson T, Jones C, Fraenkel MB, Becker G.
Vascular expression of clusterin in experimental cyclosporine nephrotoxicity.
Exp Nephrol. 1995 Jul-Aug;3(4):234-9.

59. Flach R, Cattaruzza M, Koch-Brandt C.
Clusterin gene expression in apoptotic MDCK cells is dependent on the apoptosis-inducing stimulus.
Biochim Biophys Acta. 1995 Sep 21;1268(3):325-8

60. Baumgartner-Parzer SM, Wagner L, Pettermann M, Grillari J, Gessl A, Waldhäusl W.
High-glucose--triggered apoptosis in cultured endothelial cells.
Diabetes. 1995 Nov;44(11):1323-7.

61. Koch-Brandt C, Morgans C.
Clusterin: a role in cell survival in the face of apoptosis?
Prog Mol Subcell Biol. 1996;16:130-49. Review.

62. Reddy KB, Karode MC, Harmony AK, Howe PH.
Interaction of transforming growth factor beta receptors with apolipoprotein J/clusterin.
Biochemistry. 1996 Jan 9;35(1):309-14.

63. Høgåsen K, Homann C, Mollnes TE, Graudal N, Høgåsen AK, Hasselqvist P, Thomsen AC, Garred P.
Serum clusterin and vitronectin in alcoholic cirrhosis.
Liver. 1996 Apr;16(2):140-6.

64. Arai T, Kida Y, Harmon BV, Gobé GC.
Expression and localization of clusterin mRNA in the small and large intestine of the irradiated rat: its relationship with apoptosis.
Int J Radiat Biol. 1996 May;69(5):547-53.

65. Reddy KB, Jin G, Karode MC, Harmony JA, Howe PH.
Transforming growth factor beta (TGF beta)-induced nuclear localization of apolipoprotein J/clusterin in epithelial cells.
Biochemistry. 1996 May 14;35(19):6157-63.

66. Rosemblit N, Feng ZM, Chen CL.
Analysis of the rat clusterin gene promoter and cyclic AMP-regulated mRNA stability in testicular cells.
J Mol Endocrinol. 1996 Jun;16(3):287-96

67. Swertfeger DK, Witte DP, Stuart WD, Rockman HA, Harmony JA.
Apolipoprotein J/clusterin induction in myocarditis: A localized response gene to myocardial injury.
Am J Pathol. 1996 Jun;148(6):1971-83.

68. Kimura K, Yamamoto M.
Modification of the alternative splicing process of testosterone-repressed prostate message-2 (TRPM-2) gene by protein synthesis inhibitors and heat shock treatment.
Biochim Biophys Acta. 1996 Jun 3;1307(1):83-8.

69. Morales CR, Igdoura SA, Wosu UA, Boman J, Argraves WS.
Low density lipoprotein receptor-related protein-2 expression in efferent duct and epididymal epithelia: evidence in rats for its in vivo role in endocytosis of apolipoprotein J/clusterin.
Biol Reprod. 1996 Sep;55(3):676-83.

70. Chevalier RL, Chung KH, Smith CD, Ficenec M, Gomez RA.
Renal apoptosis and clusterin following ureteral obstruction: the role of maturation.
J Urol. 1996 Oct;156(4):1474-9.

71. Brown TL, Moulton BC, Witte DP, Swertfeger DK, Harmony JA.
Apolipoprotein J/clusterin expression defines distinct stages of blastocyst implantation in the mouse uterus.
Biol Reprod. 1996 Oct;55(4):740-7.

72. Partridge SR, Baker MS, Walker MJ, Wilson MR.
Clusterin, a putative complement regulator, binds to the cell surface of Staphylococcus aureus clinical isolates.
Infect Immun. 1996 Oct;64(10):4324-9.

73. Gutacker C, Flach R, Diel P, Klock G, Koch-Brandt C.
Multiple signal transduction pathways regulate clusterin (gp 80) gene expression in MDCK cells.
J Mol Endocrinol. 1996 Oct;17(2):109-19.

74. Roeth PJ, Easterbrook-Smith SB.
C1q is a nucleotide binding protein and is responsible for the ability of clusterin preparations to promote immune complex formation.
Biochim Biophys Acta. 1996 Oct 17;1297(2):159-66.

75. Roeth PJ, Easterbrook-Smith SB.
C1q is a nucleotide binding protein and is responsible for the ability of clusterin preparations to promote immune complex formation.
Biochim Biophys Acta. 1996 Oct 17;1297(2):159-66.

76. Silkensen JR, Agarwal A, Nath KA, Manivel JC, Rosenberg ME.
Temporal induction of clusterin in cisplatin nephrotoxicity.
J Am Soc Nephrol. 1997 Feb;8(2):302-5.

77. Navab M, Hama-Levy S, Van Lenten BJ, Fonarow GC, Cardinez CJ, Castellani LW, Brennan ML, Lusis AJ, Fogelman AM, La Du BN.

Mildly oxidized LDL induces an increased apolipoprotein J/paraoxonase ratio.
J Clin Invest. 1997 Apr 15;99(8):2005-19. Erratum in: J Clin Invest 1997 Jun 15;99(12):3043.

78. Laping NJ, Olson BA, Short B, Albrightson CR.

Thrombin increases clusterin mRNA in glomerular epithelial and mesangial cells.
J Am Soc Nephrol. 1997 Jun;8(6):906-14.

79. McDonald JF, Nelsestuen GL.

Potent inhibition of terminal complement assembly by clusterin: characterization of its impact on C9 polymerization.
Biochemistry. 1997 Jun 17;36(24):7464-73.

80. Mackness B, Hunt R, Durrington PN, Mackness MI.

Increased immunolocalization of paraoxonase, clusterin, and apolipoprotein A-I in the human artery wall with the progression of atherosclerosis.
Arterioscler Thromb Vasc Biol. 1997 Jul;17(7):1233-8.

81. Jin G, Howe PH.

Regulation of clusterin gene expression by transforming growth factor beta.
J Biol Chem. 1997 Oct 17;272(42):26620-6.

82. Kapron JT, Hilliard GM, Lakins JN, Tenniswood MP, West KA, Carr SA, Crabb JW.

Identification and characterization of glycosylation sites in human serum clusterin.
Protein Sci. 1997 Oct;6(10):2120-33.

83. Lee M, Hwang I, Choi Y, Paik S, Lee HB, Baik M.

EGF inhibits expression of WDNM1 and sulfated glycoprotein-2 genes in mammary epithelial cells.
Biochem Biophys Res Commun. 1997 Nov 7;240(1):104-7.

84. Kimura K, Asami K, Yamamoto M.
Effect of heat shock treatment on the production of variant testosterone-repressed prostate message-2 (TRPM-2) mRNA in culture cells.
Cell Biochem Funct. 1997 Dec;15(4):251-7.

85. Michel D, Chatelain G, North S, Brun G.
Stress-induced transcription of the clusterin/apoJ gene.
Biochem J. 1997 Nov 15;328 (Pt 1):45-50.

86. Humphreys D, Hochgrebe TT, Easterbrook-Smith SB, Tenniswood MP, Wilson MR.
Effects of clusterin overexpression on TNFalpha- and TGFbeta-mediated death of L929 cells.
Biochemistry. 1997 Dec 9;36(49):15233-43.

87. Pampfer S, Vanderheyden I, McCracken JE, Vesela J, De Hertogh R.
Increased cell death in rat blastocysts exposed to maternal diabetes in utero and to high glucose or tumor necrosis factor-alpha in vitro.
Development. 1997 Dec;124(23):4827-36.

88. Correa-Rotter R, Ibarra-Rubio ME, Schwochau G, Cruz C, Silkensen JR, Pedraza-Chaverri J, Chmielewski D, Rosenberg ME.
Induction of clusterin in tubules of nephrotic rats.
J Am Soc Nephrol. 1998 Jan;9(1):33-7.

89. Laping NJ, Olson BA, Day JR, Brickson BM, Contino LC, Short BG, Ali SM, Brooks DP.
The age-related increase in renal clusterin mRNA is accelerated in obese Zucker rats.
J Am Soc Nephrol. 1998 Jan;9(1):38-45.

90. Cuida M, Legler DW, Eidsheim M, Jonsson R.
Complement regulatory proteins in the salivary glands and saliva of Sjögren's syndrome patients and healthy subjects.
Clin Exp Rheumatol. 1997 Nov-Dec;15(6):615-23

91. Silkensen JR, Hirsch AT, Lunzer MM, Chmielewski D, Manivel JC, Muellerleile MR, Rosenberg ME.
Temporal induction of clusterin in the peri-infarct zone after experimental myocardial infarction in the rat.
J Lab Clin Med. 1998 Jan;131(1):28-35.

92. Izawa M.
Identification of a transcript predicting an alternative form of sulfated glycoprotein-2 (clusterin) in rat tissues.
Biochem Mol Biol Int. 1998 Jan;44(1):9-18.

93. Moll S, Menoud PA, French L, Sappino AP, Pastore Y, Schifferli JA, Izui S.
Tubular up-regulation of clusterin mRNA in murine lupus-like nephritis.
Am J Pathol. 1998 Apr;152(4):953-62.

94. Gaemers IC, Van Pelt AM, Themmen AP, De Rooij DG.
Isolation and characterization of all-trans-retinoic acid-responsive genes in the rat testis.
Mol Reprod Dev. 1998 May;50(1):1-6.

95. Singhal PC, Gibbons N, Franki N, Reddy K, Sharma P, Mattana J, Wagner JD, Bansal V.
Simulated glomerular hypertension promotes mesangial cell apoptosis and expression of cathepsin-B and SGP-2.
J Investig Med. 1998 Feb;46(2):42-50.

96. Ishikawa Y, Akasaka Y, Ishii T, Komiyama K, Masuda S, Asuwa N, Choi-Miura NH, Tomita M.
Distribution and synthesis of apolipoprotein J in the atherosclerotic aorta.
Arterioscler Thromb Vasc Biol. 1998 Apr;18(4):665-72.

97. Gelissen IC, Hochgrebe T, Wilson MR, Easterbrook-Smith SB, Jessup W, Dean RT, Brown AJ.
Apolipoprotein J (clusterin) induces cholesterol export from macrophage-foam cells: a potential anti-atherogenic function?
Biochem J. 1998 Apr 1;331 (Pt 1):231-7

98. Schwochau GB, Nath KA, Rosenberg ME.
Clusterin protects against oxidative stress in vitro through aggregative and nonaggregative properties.
Kidney Int. 1998 Jun;53(6):1647-53.

99. Wünsche W, Tenniswood MP, Schneider MR, Vollmer G.
Estrogenic regulation of clusterin mRNA in normal and malignant endometrial tissue.
Int J Cancer. 1998 May 29;76(5):684-8.

100. Yoo KH, Thornhill BA, Wolstenholme JT, Chevalier RL.
Tissue-specific regulation of growth factors and clusterin by angiotensin II.
Am J Hypertens. 1998 Jun;11(6 Pt 1):715-22

101. Calvo EL, Mallo GV, Fiedler F, Malka D, Vaccaro MI, Keim V, Morisset J, Dagorn JC, Iovanna JL.
Clusterin overexpression in rat pancreas during the acute phase of pancreatitis and pancreatic development.
Eur J Biochem. 1998 Jun 1;254(2):282-9.

102. Olson BA, Ali SM, Contino LC, Brooks DP, Laping NJ.
Angiotensin-converting enzyme inhibition alters clusterin mRNA expression in the kidney following renal mass reduction.
Pharmacology. 1998 Jul;57(1):13-9.

103. Howes EA, Hurst S, Laslop A, Jones R.
Cellular distribution and molecular heterogeneity of MAC393 antigen (clusterin, beta-chain) on the surface membrane of bull spermatozoa.
Mol Hum Reprod. 1998 Jul;4(7):673-81.

104. Steinberg J, Oyasu R, Lang S, Sintich S, Rademaker A, Lee C, Kozlowski JM, Sensibar JA.
Intracellular levels of SGP-2 (Clusterin) correlate with tumor grade in prostate cancer.
Clin Cancer Res. 1997 Oct;3(10):1707-11.

105. Humphreys DT, Carver JA, Easterbrook-Smith SB, Wilson MR.
Clusterin has chaperone-like activity similar to that of small heat shock proteins.
J Biol Chem. 1999 Mar 12;274(11):6875-81.

106. Viard I, Wehrli P, Jornot L, Bullani R, Vechietti JL, Schifferli JA, Tschopp J, French LE.
Clusterin gene expression mediates resistance to apoptotic cell death induced by heat shock and oxidative stress.
J Invest Dermatol. 1999 Mar;112(3):290-6.

107. Newkirk MM, Apostolakos P, Neville C, Fortin PR.
Systemic lupus erythematosus, a disease associated with low levels of clusterin/apoJ, an antiinflammatory protein.
J Rheumatol. 1999 Mar;26(3):597-603.

108. Klock G, Storch S, Rickert J, Gutacker C, Koch-Brandt C.
Differential regulation of the clusterin gene by Ha-ras and c-myc oncogenes and during apoptosis.
J Cell Physiol. 1998 Dec;177(4):593-605.

109. Sintich SM, Steinberg J, Kozlowski JM, Lee C, Pruden S, Sayeed S, Sensibar JA.
Cytotoxic sensitivity to tumor necrosis factor-alpha in PC3 and LNCaP prostatic cancer cells is regulated by extracellular levels of SGP-2 (clusterin).
Prostate. 1999 May;39(2):87-93.

110. Ghosh P, Hale EA, Lakshman R.
Long-term ethanol exposure alters the sialylation index of plasma apolipoprotein J (Apo J) in rats.
Alcohol Clin Exp Res. 1999 Apr;23(4):720-5.

111. Hochgrebe TT, Humphreys D, Wilson MR, Easterbrook-Smith SB.
A reexamination of the role of clusterin as a complement regulator.
Exp Cell Res. 1999 May 25;249(1):13-21

112. Forte TM, Oda MN, Knoff L, Frei B, Suh J, Harmony JA, Stuart WD, Rubin EM, Ng DS.
Targeted disruption of the murine lecithin:cholesterol acyltransferase gene is associated with reductions in plasma paraoxonase and platelet-activating factor acetylhydrolase activities but not in apolipoprotein J concentration.
J Lipid Res. 1999 Jul;40(7):1276-83.

113. Park IS, Che YZ, Bendayan M, Kang SW, Min BH.
Up-regulation of clusterin (sulfated glycoprotein-2) in pancreatic islet cells upon streptozotocin injection to rats.
J Endocrinol. 1999 Jul;162(1):57-65.

114. Bailey R, Griswold MD.
Clusterin in the male reproductive system: localization and possible function.
Mol Cell Endocrinol. 1999 May 25;151(1-2):17-23. Review.

115. Lemansky P, Brix K, Herzog V.
Subcellular distribution, secretion, and posttranslational modifications of clusterin in thyrocytes.
Exp Cell Res. 1999 Aug 25;251(1):147-55.

116. Henry H, Froehlich F, Perret R, Tissot JD, Eilers-Messerli B, Lavanchy D, Dionisi-Vici C, Gonvers JJ, Bachmann C.
Microheterogeneity of serum glycoproteins in patients with chronic alcohol abuse compared with carbohydrate-deficient glycoprotein syndrome type I.
Clin Chem. 1999 Sep;45(9):1408-13.

117. Hochgrebe T, Pankhurst GJ, Wilce J, Easterbrook-Smith SB.
pH-dependent changes in the in vitro ligand-binding properties and structure of human clusterin.
Biochemistry. 2000 Feb 15;39(6):1411-9

118. Yoo KH, Thornhill BA, Chevalier RL.
Angiotensin stimulates TGF-beta1 and clusterin in the hydronephrotic neonatal rat kidney.
Am J Physiol Regul Integr Comp Physiol. 2000 Mar;278(3):R640-5

119. Cervellera M, Raschella G, Santilli G, Tanno B, Ventura A, Mancini C, Sevignani C, Calabretta B, Sala A.
Direct transactivation of the anti-apoptotic gene apolipoprotein J (clusterin) by B-MYB.
J Biol Chem. 2000 Jul 14;275(28):21055-60.

120. Kang SW, Lim SW, Choi SH, Shin KH, Chun BG, Park IS, Min BH.
Antisense oligonucleotide of clusterin mRNA induces apoptotic cell death and prevents adhesion of rat ASC-17D Sertoli cells.
Mol Cells. 2000 Apr 30;10(2):193-8.

121. Zwain I, Amato P.
Clusterin protects granulosa cells from apoptotic cell death during follicular atresia.
Exp Cell Res. 2000 May 25;257(1):101-10.

122. Chiang KC, Goto S, Chen CL, Lin CL, Lin YC, Pan TL, Lord R, Lai CY, Tseng HP, Hsu LW, Lee TH, Yokoyama H, Kunimatsu M, Chiang YC, Hashimoto T.

Clusterin may be involved in rat liver allograft tolerance.
Transpl Immunol. 2000 Jun;8(2):95-9.

---

123. Van Lenten BJ, Wagner AC, Navab M, Fogelman AM.

Oxidized phospholipids induce changes in hepatic paraoxonase and ApoJ but not monocyte chemoattractant protein-1 via interleukin-6.
J Biol Chem. 2001 Jan 19;276(3):1923-9. Epub 2000 Oct 16.

---

124. McLaughlin L, Zhu G, Mistry M, Ley-Ebert C, Stuart WD, Florio CJ, Groen PA, Witt SA, Kimball TR, Witte DP, Harmony JA, Aronow BJ.

Apolipoprotein J/clusterin limits the severity of murine autoimmune myocarditis.
J Clin Invest. 2000 Nov;106(9):1105-13.

---

125. Ibrahim NM, Gilbert GR, Loseth KJ, Crabo BG.

Correlation between clusterin-positive spermatozoa determined by flow cytometry in bull semen and fertility.
J Androl. 2000 Nov-Dec;21(6):887-94

---

126. Poon S, Easterbrook-Smith SB, Rybchyn MS, Carver JA, Wilson MR.

Clusterin is an ATP-independent chaperone with very broad substrate specificity that stabilizes stressed proteins in a folding-competent state.
Biochemistry. 2000 Dec 26;39(51):15953-60.

---

127. Yamada K, Hori Y, Hanafusa N, Okuda T, Nagano N, Choi-Miura NH, Couser WG, Miyata T, Kurokawa K, Fujita T, Nangaku M.

Clusterin is up-regulated in glomerular mesangial cells in complement-mediated injury.
Kidney Int. 2001 Jan;59(1):137-46.

---

128. Millis AJ, Luciani M, McCue HM, Rosenberg ME, Moulson CL.

Clusterin regulates vascular smooth muscle cell nodule formation and migration.
J Cell Physiol. 2001 Feb;186(2):210-9.

---

129. Atlas-White M, Murphy BF, Baker HW.
Localisation of clusterin in normal human sperm by immunogold electron microscopy.
Pathology. 2000 Nov;32(4):258-61.

130. Xie MJ, Motoo Y, Su SB, Sawabu N.
Expression of clusterin in pancreatic acinar cell injuries in vivo and in vitro.
Pancreas. 2001 Mar;22(2):126-34.

131. Bach UC, Baiersdörfer M, Klock G, Cattaruzza M, Post A, Koch-Brandt C.
Apoptotic cell debris and phosphatidylserine-containing lipid vesicles induce apolipoprotein J (clusterin) gene expression in vital fibroblasts.
Exp Cell Res. 2001 Apr 15;265(1):11-20

132. Morrissey C, Lakins J, Moquin A, Hussain M, Tenniswood M.
An antigen capture assay for the measurement of serum clusterin concentrations.
J Biochem Biophys Methods. 2001 Mar 28;48(1):13-21

133. Sivamurthy N, Stone DH, LoGerfo FW, Quist WC.
Apolipoprotein J inhibits the migration and adhesion of endothelial cells.
Surgery. 2001 Aug;130(2):204-9

134. Gassler N, Autschbach F, Heuschen G, Witzgall R, Otto HF, Obermüller N.
Expression of clusterin in Crohn's disease of the terminal ileum.
Histol Histopathol. 2001 Jul;16(3):755-62.

135. Ibrahim NM, Romano JE, Troedsson MH, Crabo BG.
Effect of scrotal insulation on clusterin-positive cells in ram semen and their relationship to semen quality.
J Androl. 2001 Sep-Oct;22(5):863-77.

136. Miyata M, Biro S, Kaieda H, Eto H, Orihara K, Kihara T, Obata H, Matsushita N, Matsuyama T, Tei C.
Apolipoprotein J/clusterin is induced in vascular smooth muscle cells after vascular injury.
Circulation. 2001 Sep 18;104(12):1407-12.

137. Bailey RW, Dunker AK, Brown CJ, Garner EC, Griswold MD.
Clusterin, a binding protein with a molten globule-like region.
Biochemistry. 2001 Oct 2;40(39):11828-40.

138. Lakins JN, Poon S, Easterbrook-Smith SB, Carver JA, Tenniswood MP, Wilson MR.
Evidence that clusterin has discrete chaperone and ligand binding sites.
Biochemistry. 2002 Jan 8;41(1):282-91.

139. Kim BM, Han YM, Shin YJ, Min BH, Park IS.
Clusterin expression during regeneration of pancreatic islet cells in streptozotocin-induced diabetic rats.
Diabetologia. 2001 Dec;44(12):2192-202.

140. Ghosh P, Hale EA, Lakshman MR.
Plasma sialic-acid index of apolipoprotein J (SIJ): a new alcohol intake marker.
Alcohol. 2001 Nov;25(3):173-9.

141. Connor JR, Kumar S, Sathe G, Mooney J, O'Brien SP, Mui P, Murdock PR, Gowen M, Lark MW.
Clusterin expression in adult human normal and osteoarthritic articular cartilage.
Osteoarthritis Cartilage. 2001 Nov;9(8):727-37.

142. Rosenberg ME, Girton R, Finkel D, Chmielewski D, Barrie A 3rd, Witte DP, Zhu G, Bissler JJ, Harmony JA, Aronow BJ.
Apolipoprotein J/clusterin prevents a progressive glomerulopathy of aging.
Mol Cell Biol. 2002 Mar;22(6):1893-902

143. Girton RA, Sundin DP, Rosenberg ME.
Clusterin protects renal tubular epithelial cells from gentamicin-mediated cytotoxicity.
Am J Physiol Renal Physiol. 2002 Apr;282(4):F703-9.

144. Dumont P, Chainiaux F, Eliaers F, Petropoulou C, Remacle J, Koch-Brandt C, Gonos ES, Toussaint O.
Overexpression of apolipoprotein J in human fibroblasts protects against cytotoxicity and premature senescence induced by ethanol and tert-butylhydroperoxide.
Cell Stress Chaperones. 2002 Jan;7(1):23-35.

145. Poon S, Treweek TM, Wilson MR, Easterbrook-Smith SB, Carver JA.
Clusterin is an extracellular chaperone that specifically interacts with slowly aggregating proteins on their off-folding pathway.
FEBS Lett. 2002 Feb 27;513(2-3):259-66.

146. Poon S, Rybchyn MS, Easterbrook-Smith SB, Carver JA, Pankhurst GJ, Wilson MR.
Mildly acidic pH activates the extracellular molecular chaperone clusterin.
J Biol Chem. 2002 Oct 18;277(42):39532-40. Epub 2002 Aug 9. Erratum in: J Biol Chem 2002 Dec 6;277(49):47964.

147. Hidaka S, Kränzlin B, Gretz N, Witzgall R.
Urinary clusterin levels in the rat correlate with the severity of tubular damage and may help to differentiate between glomerular and tubular injuries.
Cell Tissue Res. 2002 Dec;310(3):289-96. Epub 2002 Oct 2.

148. Trougakos IP, Poulakou M, Stathatos M, Chalikia A, Melidonis A, Gonos ES.
Serum levels of the senescence biomarker clusterin/apolipoprotein J increase significantly in diabetes type II and during development of coronary heart disease or at myocardial infarction.

149. Leskov KS, Klokov DY, Li J, Kinsella TJ, Boothman DA.
Synthesis and functional analyses of nuclear clusterin, a cell death protein.
J Biol Chem. 2003 Mar 28;278(13):11590-600. Epub 2003 Jan 24.

150. Park JH, Park JS, Ju SK, Lee KB, Park YK, Kang MH, Na SY, You KH.
Clusterin mRNA expression in apoptotic and activated rat thymocytes.

151. Debure L, Vayssiere JL, Rincheval V, Loison F, Le Drean Y, Michel D.
Intracellular clusterin causes juxtanuclear aggregate formation and mitochondrial alteration.
J Cell Sci. 2003 Aug 1;116(Pt 15):3109-21. Epub 2003 Jun 10.

152. Bajari TM, Strasser V, Nimpf J, Schneider WJ.
A model for modulation of leptin activity by association with clusterin.
FASEB J. 2003 Aug;17(11):1505-7. Epub 2003 Jun 3.

153. Matsuda A, Itoh Y, Koshikawa N, Akizawa T, Yana I, Seiki M.
Clusterin, an abundant serum factor, is a possible negative regulator of MT6-MMP/MMP-25 produced by neutrophils.
J Biol Chem. 2003 Sep 19;278(38):36350-7. Epub 2003 Jul 14.

154. Beaumont NJ, Skinner VO, Tan TM, Ramesh BS, Byrne DJ, MacColl GS, Keen JN, Bouloux PM, Mikhailidis DP, Bruckdorfer KR, Vanderpump MP, Srai KS.
Ghrelin can bind to a species of high density lipoprotein associated with paraoxonase.

155. Heller AR, Fiedler F, Braun P, Stehr SN, Bödeker H, Koch T.
Clusterin protects the lung from leukocyte-induced injury.
Shock. 2003 Aug;20(2):166-70

156. O'Sullivan J, Whyte L, Drake J, Tenniswood M.
Alterations in the post-translational modification and intracellular trafficking of clusterin in MCF-7 cells during apoptosis.
Cell Death Differ. 2003 Aug;10(8):914-27.

157. Min BH, Kim BM, Lee SH, Kang SW, Bendayan M, Park IS.
Clusterin expression in the early process of pancreas regeneration in the pancreatectomized rat.
J Histochem Cytochem. 2003 Oct;51(10):1355-65.

158. Lin KH, Lee HY, Shih CH, Yen CC, Chen SL, Yang RC, Wang CS.
Plasma protein regulation by thyroid hormone.
J Endocrinol. 2003 Dec;179(3):367-77.

159. Watanabe H, Hamada H, Yamada N, Sohda S, Yamakawa-Kobayashi K, Yoshikawa H, Arinami T.
Proteome analysis reveals elevated serum levels of clusterin in patients with preeclampsia.
Proteomics. 2004 Feb;4(2):537-43.

160. Forni M, Zannoni A, Tamanini C, Bacci ML.
Opposite regulation of clusterin and LH receptor in the swine corpus luteum during luteolysis.
Reprod Nutr Dev. 2003 Nov-Dec;43(6):517-25

161. Li DQ, Lundberg F, Ljungh A.
Binding of vitronectin and clusterin by coagulase-negative staphylococci interfering with complement function.
J Mater Sci Mater Med. 2001 Oct-Dec;12(10-12):979-82.

162. Orlandi A, Pucci S, Ciucci A, Pichiorri F, Ferlosio A, Spagnoli LG.
Modulation of clusterin isoforms is associated with all-trans retinoic acid-induced proliferative arrest and apoptosis of intimal smooth muscle cells.
Arterioscler Thromb Vasc Biol. 2005 Feb;25(2):348-53. Epub 2004 Dec 9.

163. Miwa Y, Takiuchi S, Kamide K, Yoshii M, Horio T, Tanaka C, Banno M, Miyata T, Sasaguri T, Kawano Y.
Insertion/deletion polymorphism in clusterin gene influences serum lipid levels and carotid intima-media thickness in hypertensive Japanese females.
Biochem Biophys Res Commun. 2005 Jun 17;331(4):1587-93.

164. Chen M, Yuan Z, Shan K.
Association of apolipoprotein J gene 866C-->T polymorphism with preeclampsia and essential hypertension.
Gynecol Obstet Invest. 2005;60(3):133-8. Epub 2005 May 27.

165. Navab M, Anantharamaiah GM, Reddy ST, Van Lenten BJ, Wagner AC, Hama S, Hough G, Bachini E, Garber DW, Mishra VK, Palgunachari MN, Fogelman AM.
An oral apoJ peptide renders HDL antiinflammatory in mice and monkeys and dramatically reduces atherosclerosis in apolipoprotein E-null mice.
Arterioscler Thromb Vasc Biol. 2005 Sep;25(9):1932-7. Epub 2005 Jun 16

166. Krijnen PA, Cillessen SA, Manoe R, Muller A, Visser CA, Meijer CJ, Musters RJ, Hack CE, Aarden LA, Niessen HW.
Clusterin: a protective mediator for ischemic cardiomyocytes?
Am J Physiol Heart Circ Physiol. 2005 Nov;289(5):H2193-202. Epub 2005 Jul 1.

167. Tsuchiya Y, Tominaga Y, Matsubayashi K, Jindo T, Furuhama K, Suzuki KT.
Investigation on urinary proteins and renal mRNA expression in canine renal papillary necrosis induced by nefiracetam.
Arch Toxicol. 2005 Sep;79(9):500-7. Epub 2005 Jul 9

168. Zhang H, Kim JK, Edwards CA, Xu Z, Taichman R, Wang CY.
Clusterin inhibits apoptosis by interacting with activated Bax.
Nat Cell Biol. 2005 Sep;7(9):909-15. Epub 2005 Aug 21.

169. Loison F, Debure L, Nizard P, le Goff P, Michel D, le Dréan Y.
Up-regulation of the clusterin gene after proteotoxic stress: implication of HSF1-HSF2 heterocomplexes.
Biochem J. 2006 Apr 1;395(1):223-31.

170. Kim BM, Kim SY, Lee S, Shin YJ, Min BH, Bendayan M, Park IS.
Clusterin induces differentiation of pancreatic duct cells into insulin-secreting cells.
Diabetologia. 2006 Feb;49(2):311-20. Epub 2006 Jan 13.

171. Nilselid AM, Davidsson P, Nägga K, Andreasen N, Fredman P, Blennow K.
Clusterin in cerebrospinal fluid: analysis of carbohydrates and quantification of native and glycosylated forms.
Neurochem Int. 2006 Jun;48(8):718-28. Epub 2006 Feb 21.

172. Plotton I, Sanchez P, Durand P, Lejeune H.
Decrease of both stem cell factor and clusterin mRNA levels in testicular biopsies of azoospermic patients with constitutive or idiopathic but not acquired spermatogenic failure

173. Carnevali S, Luppi F, D'Arca D, Caporali A, Ruggieri MP, Vettori MV, Caglieri A, Astancolle S, Panico F, Davalli P, Mutti A, Fabbri LM, Corti A.
Clusterin decreases oxidative stress in lung fibroblasts exposed to cigarette smoke.
Am J Respir Crit Care Med. 2006 Aug 15;174(4):393-9. Epub 2006 May 18.

174. Rastaldi MP, Candiano G, Musante L, Bruschi M, Armelloni S, Rimoldi L, Tardanico R, Sanna-Cherchi S, Ferrario F, Montinaro V, Haupt R, Parodi S, Carnevali ML, Allegri L, Camussi G, Gesualdo L, Scolari F, Ghiggeri GM.
Glomerular clusterin is associated with PKC-alpha/beta regulation and good outcome of membranous glomerulonephritis in humans.
Kidney Int. 2006 Aug;70(3):477-85. Epub 2006 Jun 14.

175. Stejskal D, Fiala RR.
Evaluation of serum and urine clusterin as a potential tumor marker for urinary bladder cancer.
Neoplasma. 2006;53(4):343-6.

176. Chauhan AK, Moore TL.
Presence of plasma complement regulatory proteins clusterin (Apo J) and vitronectin (S40) on circulating immune complexes (CIC).
Clin Exp Immunol. 2006 Sep;145(3):398-406

177. Devauchelle V, Essabbani A, De Pinieux G, Germain S, Tourneur L, Mistou S, Margottin-Goguet F, Anract P, Migaud H, Le Nen D, Lequerré T, Saraux A, Dougados M, Breban M, Fournier C, Chiocchia G.
Characterization and functional consequences of underexpression of clusterin in rheumatoid arthritis.
J Immunol. 2006 Nov 1;177(9):6471-9.

178. Kujiraoka T, Hattori H, Miwa Y, Ishihara M, Ueno T, Ishii J, Tsuji M, Iwasaki T, Sasaguri Y, Fujioka T, Saito S, Tsushima M, Maruyama T, Miller IP, Miller NE, Egashira T.
Serum apolipoprotein j in health, coronary heart disease and type 2 diabetes mellitus.
J Atheroscler Thromb. 2006 Dec;13(6):314-22.

179. Savković V, Gantzer H, Reiser U, Selig L, Gaiser S, Sack U, Klöppel G, Mössner J, Keim V, Horn F, Bödeker H.
Clusterin is protective in pancreatitis through anti-apoptotic and anti-inflammatory properties.
Biochem Biophys Res Commun. 2007 May 4;356(2):431-7. Epub 2007 Mar 6

180. Li Y, Sagar MB, Wassler M, Shelat H, Geng YJ.
Apolipoprotein-J prevention of fetal cardiac myoblast apoptosis induced by ethanol.
Biochem Biophys Res Commun. 2007 May 25;357(1):157-61. Epub 2007 Mar 28.

181. Nizard P, Tetley S, Le Dréan Y, Watrin T, Le Goff P, Wilson MR, Michel D.
Stress-induced retrotranslocation of clusterin/ApoJ into the cytosol.
Traffic. 2007 May;8(5):554-65.

182. Ishii A, Sakai Y, Nakamura A.
Molecular pathological evaluation of clusterin in a rat model of unilateral ureteral obstruction as a possible biomarker of nephrotoxicity.
Toxicol Pathol. 2007;35(3):376-82.

183. Yon JM, Kwak DH, Cho YK, Lee SR, Jin Y, Baek IJ, Lee JE, Nahm SS, Choo YK, Lee BJ, Yun YW, Nam SY.
Expression pattern of sulfated glycoprotein-2 (SGP-2) mRNA in rat testes exposed to endocrine disruptors.
J Reprod Dev. 2007 Oct;53(5):1007-13. Epub 2007 Jun 8

184. Schepeler T, Mansilla F, Christensen LL, Orntoft TF, Andersen CL.
Clusterin expression can be modulated by changes in TCF1-mediated Wnt signaling.
J Mol Signal. 2007 Jul 16;2:6.

185. Kurosu T, Chaichana P, Yamate M, Anantapreecha S, Ikuta K.
Secreted complement regulatory protein clusterin interacts with dengue virus nonstructural protein 1.
Biochem Biophys Res Commun. 2007 Nov 3;362(4):1051-6. Epub 2007 Aug 30

186. Kim JH, Yu YS, Kim JH, Kim KW, Min BH.
The role of clusterin in in vitro ischemia of human retinal endothelial cells.
Curr Eye Res. 2007 Jul-Aug;32(7-8):693-8.

187. Janig E, Haslbeck M, Aigelsreiter A, Braun N, Unterthor D, Wolf P, Khaskhely NM, Buchner J, Denk H, Zatloukal K.
Clusterin associates with altered elastic fibers in human photoaged skin and prevents elastin from ultraviolet-induced aggregation in vitro.
Am J Pathol. 2007 Nov;171(5):1474-82. Epub 2007 Sep 14.

188. Janig E, Haslbeck M, Aigelsreiter A, Braun N, Unterthor D, Wolf P, Khaskhely NM, Buchner J, Denk H, Zatloukal K.
Clusterin associates with altered elastic fibers in human photoaged skin and prevents elastin from ultraviolet-induced aggregation in vitro.
Am J Pathol. 2007 Nov;171(5):1474-82. Epub 2007 Sep 14.

189. Takase O, Minto AW, Puri TS, Cunningham PN, Jacob A, Hayashi M, Quigg RJ.
Inhibition of NF-kappaB-dependent Bcl-xL expression by clusterin promotes albumin-induced tubular cell apoptosis.
Kidney Int. 2008 Mar;73(5):567-77. Epub 2007 Dec 12.

190. Lee KB, Jeon JH, Choi I, Kwon OY, Yu K, You KH.
Clusterin, a novel modulator of TGF-beta signaling, is involved in Smad2/3 stability.
Biochem Biophys Res Commun. 2008 Feb 22;366(4):905-9. Epub 2007 Dec 17.

191. Metz TO, Qian WJ, Jacobs JM, Gritsenko MA, Moore RJ, Polpitiya AD, Monroe ME, Camp DG 2nd, Mueller PW, Smith RD.
Application of proteomics in the discovery of candidate protein biomarkers in a diabetes autoantibody standardization program sample subset.
J Proteome Res. 2008 Feb;7(2):698-707. Epub 2007 Dec 20.

192. Ammar H, Closset JL.
Clusterin activates survival through PI-3 kinase/AKT pathway.
J Biol Chem. 2008 Mar 5; [Epub ahead of print]

193. Stoop MP, Dekker LJ, Titulaer MK, Burgers PC, Sillevis Smitt PA, Luider TM, Hintzen RQ.
Multiple sclerosis-related proteins identified in cerebrospinal fluid by advanced mass spectrometry.
Proteomics. 2008 Mar 20; [Epub ahead of print]

194. Duguid JR, Bohmont CW, Liu NG, Tourtellotte WW.
Changes in brain gene expression shared by scrapie and Alzheimer disease.
Proc Natl Acad Sci U S A. 1989 Sep;86(18):7260-4.

195. Pasinetti GM, Finch CE.
Sulfated glycoprotein-2 (SGP-2) mRNA is expressed in rat striatal astrocytes following ibotenic acid lesions.
Neurosci Lett. 1991 Sep 2;130(1):1-4

196. Jones SE, Meerabux JM, Yeats DA, Neal MJ.
Analysis of differentially expressed genes in retinitis pigmentosa retinas. Altered expression of clusterin mRNA.
FEBS Lett. 1992 Apr 6;300(3):279-82.

197. Michel D, Chabot JG, Moyse E, Danik M, Quirion R.
Possible functions of a new genetic marker in central nervous system: the sulfated glycoprotein-2 (SGP-2).
Synapse. 1992 Jun;11(2):105-11. Review.

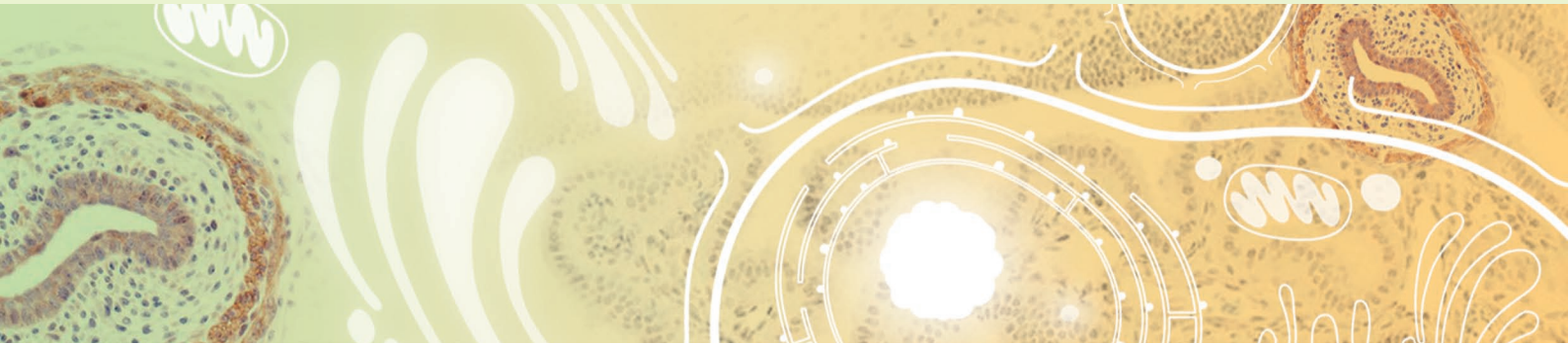
198. May PC, Robison P, Fuson K, Smalstig B, Stephenson D, Clemens JA.
Sulfated glycoprotein-2 expression increases in rodent brain after transient global ischemia.
Brain Res Mol Brain Res. 1992 Sep;15(1-2):33-9.

199. May PC, Robison P, Fuson K, Smalstig B, Stephenson D, Clemens JA.
Sulfated glycoprotein-2 expression increases in rodent brain after transient global ischemia.
Brain Res Mol Brain Res. 1992 Sep;15(1-2):33-9

200. Watari H, Ohta Y, Hassan MK, Xiong Y, Tanaka S, Sakuragi N.
Clusterin expression predicts survival of invasive cervical cancer patients treated with radical hysterectomy and systematic lymphadenectomy.
Gynecol Oncol. 2008 Mar;108(3):527-32. Epub 2008 Jan 4.

201. Redondo M, Téllez T, Roldan MJ, Serrano A, García-Aranda M, Gleave ME, Hortas ML, Morell M.
Anticlusterin treatment of breast cancer cells increases the sensitivities of chemotherapy and tamoxifen and counteracts the inhibitory action of dexamethasone on chemotherapy-induced cytotoxicity.
Breast Cancer Res. 2007;9(6):R86.

202. Miyake H, Hara I, Fujisawa M, Gleave ME.
The potential of clusterin inhibiting antisense oligodeoxynucleotide therapy for prostate cancer.
Expert Opin Investig Drugs. 2006 May;15(5):507-17. Review.



CONTACTS:

BioVendor Laboratory Medicine, Inc.
CTPark Modrice
Evropska 873
664 42 Modrice
Czech Republic
Phone: +420-549 124 185
Fax: +420-549 211 460
E-mail: info@biovendor.com
www.biovendor.com

European Union

BioVendor GmbH
Im Neuenheimer Feld 583
D-69120 Heidelberg
Germany
Phone: +49-6221-433-9100
Fax: +49-6221-433-9111

USA, Canada and Mexico

BioVendor, LLC
1463 Sand Hill Road
Suite 227
Candler, NC 28715
USA
Phone: +1-828-670-7807
Phone: +1-800-404-7807
Fax: +1-828-670-7809

China

BioVendor Laboratories Ltd
Room 2405
YiYa Tower
TianYu Garden, No.150
Lihe Zhong Road
Guang Zhou
Mainland CHINA
Phone: +86-20-38840399
Phone: +86-20-38840386
Fax: +86-20-38840386

China – Hong Kong

BioVendor Laboratories Ltd
Room 4008
Hong Kong Plaza, No. 188
Connaught Road West
Hong Kong
CHINA
Phone: +852-28030523
Fax: +852-28030525

BioVendor
Research
and Diagnostic Products